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BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

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PHYSIOLOGY

THE EFFECT OF REMOVAL OF THE PANCREAS AND ADMINISTRATION OF ACETYLCHOLINE ON THE PERIPHERAL DIVISIONS OF THE REFLEX ARC IN COLD-BLOODED ANIMALS

L. N. Zefirov and G. I. Poletaev

From the Department of Physiology (Head — Dr. Med. Sci. I. N. Volkova) of the Kazan' Medical Institute

(Received July 2, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The pancreas plays an important role in the activity of the body. The evidence which we have goes to show that, besides its well-known importance in carbohydrate metabolism, it takes part in phospholipid metabolism [7, 11, 12] and in the associated acetylcholine metabolism [5].

It was shown in previous research [1, 2, 3] that interference with acetylcholine metabolism by means of removal of the pancreas causes a diminution of some of the functional properties of the neuromuscular apparatus. Administration of pharmacological acetylcholine prevented the development of these changes. These experiments were performed exclusively with the use of myographic recording.

In the present communication we give the results of electrophysiological investigations of the common nerve trunk, the myoneural junction and the proprioceptors of the skeletal muscles of the frog after removal of the pancreas and administration of small doses of acetylcholine to the animal after operation.

EXPERIMENTAL METHOD

The research was carried out on a nerve-muscle preparation of the gastrocnemius muscle and the sciatic nerve, and on the sciatic-tibial nerve trunk of the frog (Rana ridibunda) on the 5th-9th day after pancreatectomy. Acetylcholine was injected daily, starting on the 3rd day after operation, into the posterior lymph sac (0.5 ml of a 1:10,000 solution). Stimulation of the motor nerve was effected by means of an electronic device. The action currents of the nerve and muscle were recorded on a two-channel cathode oscillograph. Altogether 185 experiments were performed, including 110 to investigate the nerve trunk, 25 the myoneural junction and 50 the proprioceptors. In a series of experiments all the investigations were carried out on a single nerve-muscle preparation. In these cases the proprioceptors were studied first, followed by the myoneural junction and lastly, by the nerve trunk.

EXPERIMENTAL RESULTS

After removal of the pancreas the following changes were observed in the activity of the above-mentioned elements of the somatic nervous system.

a) Investigations on the nerve trunk. The development and spread of a wave of excitation in the nerve were preserved for different lengths of time after the operation. The degree of excitation was altered insignificantly and irregularly. In some cases an increase in the excitation of the nerve trunk was also observed. The velocity of spread of the waves of excitation in the most rapidly conducting fibers (A,α) fell slightly, and on the 6th-9th day after operation its average value was 28.5 m/sec instead of 32 m/sec in the animals not subjected to operation (Fig. 1 a, b). The velocity of conduction of excitation in the other groups of nerve fibers

was not determined, but drawing out and splitting up of the action currents were systematically observed (Fig. 1, b), demonstrating that the velocity of spread of excitation in the more slowly conducting fibers (β) had decreased to an even greater extent.

The optimal rate of excitation, determined by the lowest frequency causing a reduction in the action currents of the nerve [6], fell to 50-70 per second (normal 80-150 per second). The limits of transformation of the action currents (maximum rhythm) were also displaced toward lower frequencies and had values of 210-270 per second (normal 400-450 per second) (Fig. 1 c, d).

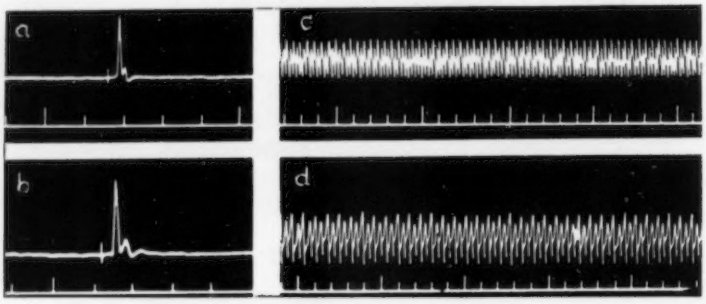

Fig. 1. Action currents in the sciatic nerve of the frog. Velocity of spread of the impulse in a normal frog (a) and in a frog on which the operation was performed 7 days before the experiment (b). Limits of transformation (alternating rhythm) in a normal frog (c) and in a frog undergoing operation 7 days before the experiment (d). Frequency of stimulation 550 and 270 per second respectively. Time marker 0.01 second.

A decrease thus took place in the lability of the nerve trunk when determined both at the optimum and the maximum frequency of stimulation. As has been shown by myographic and electrophysiological investigations of single tetanic contractions [4, 9] carried out under similar conditions, this decrease in lability was associated with a strengthening and drawing out of the subsequent processes.

b) Investigations on the motor nerve endings. Direct determination of the lability of the myoneural junction by an electrophysiological method showed that transformation and a lowering of the amplitude of the action currents of the muscle were observed even at a frequency of 35-50 stimuli per second. Complete disappearance of the electrical activity (pessimum) took place at frequencies of stimulation of 60-80 instead of 100-110 per second in the unoperated animals. The stability of the myoneural junction was sharply reduced. In response to tetanic stimulation of the motor nerve at a frequency of 35 per second, the action currents of the muscle completely disappeared after only 5-15 seconds of stimulation (in the unoperated animals after 40 seconds and over). These findings of a decrease in the lability of the myoneural junction after removal of the pancreas confirmed the results of experiments on warm-blooded animals previously described by us [3].

c) Investigations on the proprioceptors. The degree of excitation of the proprioceptors, determined by the magnitude of the stretching of the muscle to cause minimum electrical activity of the nerve connected with the muscle, fell slightly (average value in normal conditions 3.3 g, after removal of the pancreas 4.2 g). The frequency of impulsation depended on the load, and when this was twice the threshold weight the average frequency was 108 impulses per second (120 in the unoperated frogs), and in response to a standard 100 gram stretching, on the average 191 impulses per second (232 in the unoperated frogs). A fall in the adaptation of the receptors was also observed. The frequency of impulsation at the end of the first minute of stretching of the muscle in response to twice the threshold load was 31.8% of the initial value, and in response to a standard 100 gram load, 84.6% (in the unoperated animals 27.7 and 75% respectively). The course of adaptation was altered: in the unoperated frogs the greatest fall in the frequency of impulsation was observed in the first 10 seconds of stretching, but after removal of the pancreas, at 30 seconds (Fig. 2 a, b).

d) The effect of acetylcholine. The systematic injection of small doses of acetylcholine to the animals after operation led to normalization of the functional state of the neuromuscular apparatus. Under these circumstances an obvious fall in the excitation of the nerve trunk was observed. Experiments carried out at the same

time (in February) showed that after removal of the pancreas the degree of excitation corresponded, on the average, to 0.5 of a scale division of the stimulator, and after the animal had received an injection of acetylcholine, to 1.4 divisions. An increase also took place in the resistance of the nerve to alteration and in the optimum and maximum frequencies to 100-150 and 300-400 stimuli per second respectively. At the same time a diminution of the aftereffects was observed, as shown by disappearance of the ability to develop single tetanic responses.

The resistance of the myoneural junction to tetanic stimulation was increased. The limits of the pessimum and of the disappearance of the action currents of the muscle were displaced toward higher frequencies. The adaptation of the proprioceptors was accelerated, its value at the end of the first minute of stretching by twice the threshold load being 23%, and by a 100-gram load 72% of the original frequency. The adaptation curve acquired the typical character of the unoperated animals (Fig. 2, c). Thus after removal of the pancreas, the character of the proprioceptive impulsation became close to the impulsation of muscles in tetany, and after injection of acetylcholine to the impulsation of the proprioceptors of tonic muscles [8].

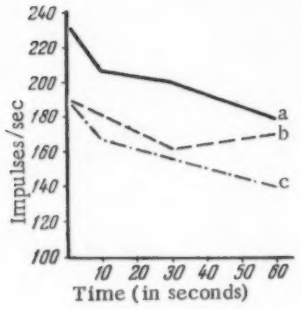

Fig. 2. Curves of adaptation of the proprioceptors of the gastrocnemius muscle of the frog. a) Unoperated; b) after removal of the pancreas; c) in response to the compensatory injection of acetylcholine to the operated animal. Along the abscissa — time of stretching, in seconds, along the ordinate axis — frequency of impulsation. Load 100 g.

It must be pointed out, however, that clear differences were observed in the reaction of the elements of the reflex arc to administration of acetylcholine. Whereas in the nerve trunk, the injected acetylcholine behaved almost exclusively as a factor enhancing its functional state, overdosage of the drug had a pessimal effect on the nerve endings.

The results obtained showed that removal of the pancreas affected the functions of the various elements of the somatic nervous system to a different degree. It caused more substantial changes in the nerve and myoneural junction, and less so in the receptor apparatus of the skeletal muscles.

The electrophysiological investigations carried out provided direct proof of the fact that after removal of the pancreas the waves of excitation in the elements of the somatic nervous system became drawn out, and a stagnating parabiotic process developed in them, characterized by diminished lability, unchanged or even increased excitation, decreased velocity of conduction of the nerve impulse and lowered adaptation of the receptors. The systematic administration of acetylcholine to animals after operation produced an increase in the functional properties and normalization of the functional state of the nerve tissue.

The results obtained confirmed A. A. Ukhtomskii's hypothesis [10] that in the somatic nervous system acetylcholine is a regulator of the functional state of the nerve tissue, and can thus be regarded as a trophic factor of the somatic nervous system [1].

SUMMARY

Preliminary (several days before the experiments) removal of the pancreas in frogs caused a certain decrease in the rate of the excitation spread and reduction of the nerve stem lability. Both the lability and the stability of the myoneural connection are decreased as well as the excitability, the frequency of impulsation and adaptation of the proprioceptors of the neuromuscular appratus. Systematic administration of acetylcholine to the operated animals prevents the development of the mentioned changes in the preparation.

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*Original Russian pagination. See C.B. Translation.

THE ROLE OF THE ACETYLCHOLINE – CHOLINESTERASE SYSTEM IN THE ORIGIN OF BIOELECTRICAL PHENOMENA IN SKELETAL MUSCLE

A. S. Mozzhukhin (Leningrad)

(Received December 3, 1956. Presented by Academician the late L. A. Orbeli)

In 1938 A. G. Ginetsinskii and N. I. Mikhel'son [1] put forward the view that a connection existed between the state of the acetylcholine in a muscle and the bioelectrical phenomena. These authors found that direct stimulation of a muscle poisoned with eserine led in addition to the development of a contracture, to an increasing negative electrical potential. The action currents gradually diminished and reached negligible values.

They came to the conclusion that "the widespread idea of a single mechanism lying at the basis of the production of the injury potential and the action potential is illustrated very expressively by the electrical phenomena in a muscle poisoned with eserine, which may be regarded as something occupying a middle position between the action current and the demarcation current".

In the same year Kh. S. Koshtoyants put forward the hypothesis of a connection between the synthesis of acetylcholine and the carbohydrate-phosphorus metabolism, which was further developed in a series of subsequent investigations [4, 5, 6, 7, and others]. On the basis of these findings, Kh. S. Koshtoyants came to the conclusion that the bioelectrical phenomena were connected in their origin with the state of the carbohydrate-phosphorus metabolism and with the state of the acetylcholine – cholinesterase system [5, 6, 7 and others].

During the period 1941-1947, Nachmansohn and his co-workers developed the theory of the relationship between the action currents of different tissues (nerve muscle, the electric organ of fishes) and the state of the acetylcholine–cholinesterase system.

From a summing up of the experimental data, Nachmansohn and his co-workers [11] reported that the view of the role of acetylcholine in the origin of the bioelectrical phenomena was based: 1) on the presence of a high concentration of cholinesterase in nerves, which shows that large amounts of acetylcholine must be hydrolyzed in the course of a millisecond; 2) on findings that cholinesterase is localized in the superficial layer of the nerve; and 3) on the close parallel between the concentration of cholinesterase and the maximum emf of the action current. By direct measurements [12] they showed the proportional relationship between the cholinesterase activity and the magnitude of the discharge of the electric organ.

These results were confirmed later both by experiments in which nerve and muscle were poisoned with eserine and strychnine, paralyzing the cholinesterase [8, 9] and by experiments with DFP (di-isopropylfluorophosphate), which also inactivates cholinesterase [9].

These authors showed that strychnine in a concentration of 0.0005 and 0.001 M and eserine in concentrations of 0.01, 0.005 and 0.02 M lead to disappearance of the action currents of nerve and muscle. This was explained by the fact that strychnine and eserine are inhibitors of cholinesterase, with the result that after the administration of these drugs, the acetylcholine secreted at the moment of excitation gives a persistently negative potential to the membranes, for it does not undergo hydrolysis for a long time.

These hypotheses, however, aroused a number of objections both in relation to the DFP experiments and in relation to experiments with eserine, strychnine and proserine [2, 10].

S. D. Kovtun [3] came to the conclusion that acetylcholine cannot be the agent which directly stimulates the process of excitation in nerves and muscles, for during its action on a nerve, electrical motor phenomena are not generally observed, and during its action on muscle, the negative potential reached only 10-20 mv, whereas during the process of excitation the negative potential reached 35-60 mv.

Certain differences of opinion thus exist in our understanding of the role of the acetylcholine—cholinesterase system in the generation of action currents, and there are discrepancies in the experimental findings on this subject.

Our experiments were undertaken in order to study the role of the acetylcholine—cholinesterase system in the origin of bioelectrical phenomena.

TABLE 1

The Effect of Strychnine ($1:10^4$) and Eserine ($1:10^4$) on the emf of Injury of the Skeletal Muscle of the Frog (mean of 5 experiments)

Time from beginning of expt. (in hours)	emf in % of initial value during the action of				
	control	on the injured portion of the muscle		on the uninjured portion of the muscle	
		eserine	strychnine	eserine	strychnine
1	80	84	81	86	83
2	74	76	71	80	75
3	68	74	66	78	67
4	65	67	64	72	65
5	62	65	62	71	63
6	59	59	57	62	58

TABLE 2

The Effect of Strychnine ($1:10^3$) and Eserine ($1:10^3$) on the emf of Injury of the Skeletal Muscle of the Frog (mean of 5 experiments)

Time from beginning of expt. (in hours)	emf in % of initial value during the action of				
	control	on the injured portion of the muscle		on the uninjured portion of the muscle	
		eserine	strychnine	eserine	strychnine
1	86	90	80	82	81
2	81	82	80	76	57
3	76	77	73	75	52
4	73	72	71	72	38
5	72	68	66	70	37
6	63	64	66	67	31

EXPERIMENTAL METHOD

Experiments were carried out on the isolated sartorius muscles of autumn and winter frogs. We measured the rate of fall of the electromotive force (emf) of injury and the changes in the value of the emf of excitation under the influence of the action of solutions of eserine in concentrations of $1:10^4$ and $1:10^5$ and of strychnine, $1:10^3$, $1:10^4$ and $1:10^5$.

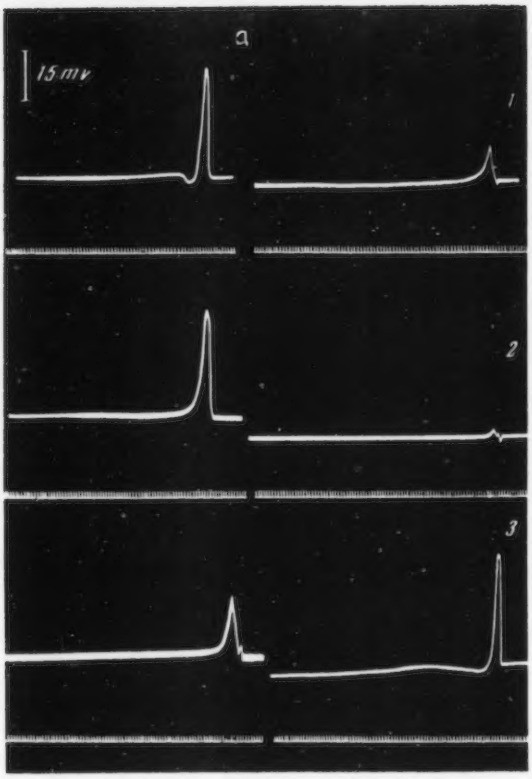

Fig. 1. The effect of poisoning an isolated muscle with strychnine ($1:10^4$) on the emf of excitation. A: 1) Initial tracing; 2) after a stay of 15 minutes in the solution; 3) after 30 minutes. B: 1) after a stay of 45 minutes in the same solution; 2) after 60 minutes; 3) the opposite muscle after a stay of 60 minutes in Ringer's solution. Time marker 2 milliseconds.

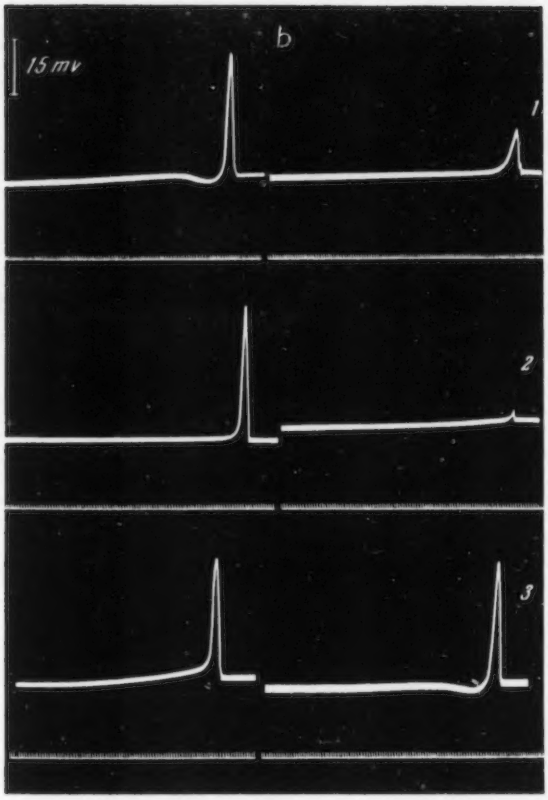

Fig. 2. The effect of poisoning an isolated muscle with eserine ($1:10^4$) on the emf of excitation. Legend as in Fig. 1.

In order to measure the emf of injury, the pelvic end of the sartorius muscle was crushed by means of a special clamp and the muscle was placed in a paraffin wax bath so that its injured end was in one compartment and the uninjured end in the other. Between these compartments a partition was created from a mixture of paraffin wax and paraffin oil. Into each compartment of the bath, Ringer's solution or one of the test solutions was poured at the time of the experiment.

The emf of injury was measured by a compensation method, using ordinary ($Zn-ZnSO_4$) nonpolarizing electrodes with agar taps. In all,40 such experiments were performed.

During the recording of the emf of excitation the muscle was kept on a wax block in a humid chamber. To avoid distortion of the tracing by contraction, the muscle was fixed in a slightly stretched position.

The excitation current was led from the injured and uninjured areas of the muscle by means of nonpolarizing electrodes to a loop oscillograph (loop T-5). When such leads were used the current was always monophasic.

Direct stimulation of the muscle was carried out by impulses of current above threshold value, with a duration of 10 milliseconds and at intervals of 1 second. The distance between the platinum stimulating electrodes was 1-1.5 mm, the distance between the stimulating and the lead electrodes was 10 mm, and that between the lead electrodes 5-10 mm.

In all,20 experiments were carried out, in 10 of which the muscle was poisoned with strychnine and in 10 with eserine.

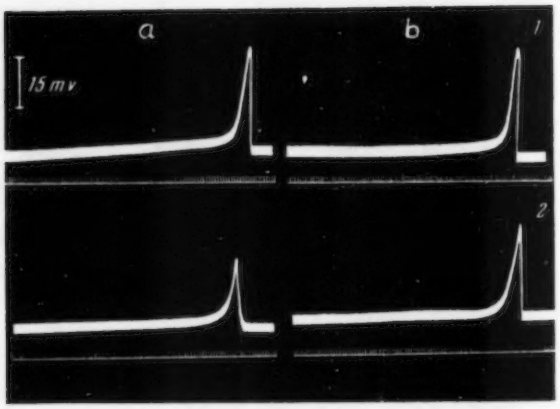

Fig. 3. The effect of poisoning an isolated muscle with eserine – 1 ($1:10^5$), with strychnine – 2 ($1:10^5$) on the emf of excitation. A) After a stay of 60 minutes in the solution; B) initial state. Time marker 2 milliseconds.

EXPERIMENTAL RESULTS

In Table 1 are shown the results of experiments with changes in the emf of injury, showing that strychnine and eserine, in dilutions lower than those used by Nachmansohn, had no appreciable effect on the emf of injury, except in cases where strychnine acted on the uninjured segment of the muscle.

In Figs. 1 and 2 are shown the results of experiments on the effect of eserine and strychnine on the value of the emf of excitation, from which it can be seen that eserine and strychnine in concentrations of $1:10^4$ caused obvious changes in this emf. Under these circumstances strychnine produced more considerable changes in the magnitude and duration of the peak of the emf of excitation.

Fig. 3 illustrates the changes in the emf of excitation during poisoning of the muscle with $1:10^5$ solutions of strychnine and eserine. As is seen from Fig. 3, strychnine, in this concentration, causes comparatively small changes in the emf of excitation, while eserine produces practically no change.

The experiments described do not support the conclusions of A. G. Ginetsinskii and N. I. Mikhel'son that the mechanism of origin ofthe injury and excitation currents in skeletal muscle is the same.

According to our findings, strychnine and eserine are capable of producing changes in the magnitude and duration of the current of excitation, but have practically no effect on the magnitude nor the rate of fall of the injury current, as is particularly clearly seen after poisoning the muscle with eserine.

From the findings described above, certain differences may thus be discerned in the mechanism of origin of the currents of the injury and excitation in skeletal muscle, at least in respect to the participation of the acetylcholine–cholinesterase system in this process.

SUMMARY

Poisoning of the isolated sartorius muscle in frog with eserine and strychnine in the $1:10^5$ and $1:10^4$ concentrations causes the change of the value and the form of the excitation biocurrent. Strychnine and eserine in the $1:10^5$, $1:10^4$ and $1:10^3$ concentrations have no effect on the electromotive force of the sartorius muscle injury. These data lead us to presume certain differences in the mechanism of the origin of the excitation and injury biocurrents in the skeletal muscle, at least with relation to the participation of the acetylcholine–cholinesterase system in this process.

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THE REFLEX CONTROL OF THE CARDIAC ACTIVITY IN FISHES

COMMUNICATION II. REFLEX EFFECTS ON THE HEART AND INTESTINAL VESSELS IN RESPONSE TO STIMULATION OF THE PRESSURE RECEPTORS OF THE BRANCHIAL VESSELS

I. M. Rodionov

From the Department of Animal Physiology (Head—Corresponding Member AMN SSSR Prof. Kh. S. Koshtoyants) of the M. V. Lomonosov Moscow State University

(Received August 19, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The heart of fishes, in contrast to that of other vertebrates, has only one pathway of effector innervation, the vagus nerve. The fish is therefore an object of peculiar interest in the study of the regulating effect of this nerve on the heart. There is evidence that no inhibitory vagotonic action on the heart is present in the fish [1, 3]. Any increase in the heart rate arising as the result of nervous action must, therefore, be regarded as the result of an active impulsive influence of the vagus nerve on the heart. It was shown in the previous communication [3] that both inhibitory and stimulatory effects could be obtained reflexly from the same receptive field (the receptors of the intestine), and moreover that the stimulatory effect is the result of weaker afferent stimulation than the inhibitory. These findings support the view [4, 5] that active impulses, passing along the vagus nerve, may exert different effects—inhibitory and stimulatory—on the action of the heart.

There is experimental evidence in the literature of the presence of receptors in the branchial vessels of the fish [9, 11, 12]. It has been shown [2] that in response to stimulation of the branchial nerves, depending on the number of fibers stimulated and on the strength of stimulation, both a slowing and a quickening of the rhythm of the cardiac action may develop.

This communication is devoted to further investigation of the controlling influences exerted on the heart of fishes, depending on the intensity of afferent stimulation.

Stimuli were applied to the pressure receptors situated in the abdominal aorta and the afferent branchial vessels. The choice of this particular reflexogenic zone was made not only on account of the relative ease of adequate stimulation and variation of the intensity of stimulation of the receptors situated therein, but also because the branchial vessels, so far as their sensory apparatus is concerned, are the homologs of such reflexogenic zones as the aorta and carotid sinus [6, 10].

EXPERIMENTAL METHOD

Experiments were carried out on bony fishes — sheat-fish, carp and tench (35 experiments), — and also on two species of ray-fish — the long-tailed skate and sea-fox (12 experiments). Fixation of the fishes and recording of the cardiac contractions were carried out as described in the first communication [3]. In the dissection of the branchial vessels, the skin over the lower jaw was incised along the midline. The connective tissue film covering the abdominal aorta and the afferent branchial vessels was exposed and divided. A cannula was inserted into the peripheral end of the vessel. The corresponding efferent branchial vessel was ligated. The cannula was connected to a burette, filled with Ringer's solution, which was used to distend the vessel. The pressure was calculated in cm of water. After the conclusion of stimulation the pressure in the vessel was made equal to

atmospheric. In addition to the reflex effects on the heart from the branchial vessels, we also recorded the velocity of the blood flow through the intestinal vessels and the movements of the gill coverings.

In order to record the vascular effects an area of the small intestine of the fishes was perfused with Samoilov's solution. The nerve supply of the perfused area was left intact. The frequency of falling of drops of perfusing fluid flowing from the vein was recorded. All the experiments of this series were performed on sheat-fish.

EXPERIMENTAL RESULTS

The experiments showed that during distension of the branchial vessels with varying force, both stimulatory and inhibitory changes could be produced reflexively in the action of the heart. The character of the response did not depend on which of the five branchial vessels was distended. Biphasic reflex responses could develop in the heart from any of them. Inhibition of the heart's rhythm arose, as a rule, in response to stronger stimulation than that producing an increase in the rate of the heart. Fig. 1 illustrates an experiment in which distension of the vessel with a pressure of 11 cm of water caused a sharp slowing of the rate of the heart, and with a pressure of 6 cm, a quickening of the rate.

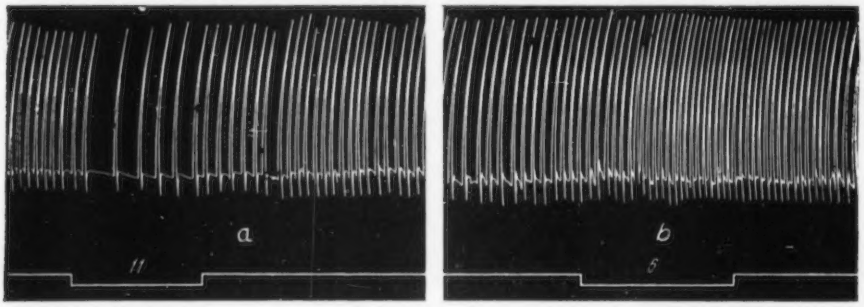

Fig. 1. Experiment on a long-tailed skate. Different forms of reflex change in the rhythm of contraction of the heart with increase in the pressure in the first branchial vessel to 11 cm (a) and to 6 cm (b) of water.

Weak stimulation evidently activates a smaller number of sensory endings than stronger. Discharge of impulses from each individual receptor is also, probably, more frequent in response to stronger than to weaker stimulation. In view of these considerations it may be thought that the reflex inhibition of the cardiac activity in response to stretching of the receptors of the branchial vessels arose as the result of a large number of impulses passing to the central nervous system, and that a smaller number caused the opposite effect, an increase in the rate of the heart.

We found this relationship described between the strength of distension of the branchial vessel and the character of the reflex response of the heart in several species of fishes, in the 2 species of ray-fish, carp, crucians and sheat-fish.

In Fig. 2 are shown the results of one of the experiments on the sheat-fish. Stretching with a force of 40 cm was accompanied by a slowing of the heart rate, whereas a smaller stretching force caused a sharp increase in the rate of contraction of the heart, giving place, very soon, to inhibition. This secondary effect was evidently due to overstretching of the remaining branchial vessels in consequence of the quickening of the heart's action.

Depending on the degree of afferent influences from the branchial vessels, both stimulatory and inhibitory effects may thus be reflexively produced on the heart. Bearing in mind the information given in the previous communication, it must be realized that both effects arose as a result of the effect of an active flow of impulses along the vagus nerve to the heart. The character of the response, i.e., stimulation or inhibition of the rhythmic action of the heart by reflex excitation, was dependent on the number of afferent impulses reaching the central nervous system in unit time.

It is easy to understand the adaptive importance of the relationship described between the pressure and the changes in the working of the heart. In fact, a low pressure in the vessel is a signal of inadequate filling of the abdominal aorta with blood, creating unsatisfactory blood supply conditions for the tissues. The adaptive response to this is an increase in the heart rate and a more intensive redistribution of blood from the venous to the arterial part of the blood stream. A high pressure in the vessels, i.e., overfilling of the branchial vessels with blood, on the other hand, causes inhibition of the cardiac activity and the temporary diminution or cessation of the entry of blood into those vessels.

The function of the pressure receptor zone of the branchial vessels of fishes differs in certain features from that of the homologous zones of the carotid sinus and aorta. If, when the abdominal aorta is well filled with blood, the conus arteriosus of the fish is divided, and the branchial vessels thereby completely emptied, no stable or considerable changes in the rate of the heart are induced. The pressure receptors of these vessels in fishes do not, therefore, exercise a constant control over the level of the blood pressure, as occurs in the warm-blooded animals [7, 8]. It is possible that the absence of tonic reflex influences from the branchial vessels of fishes during their prolonged distension is due to the rapid adaptation of the receptors of this reflexogenic zone to a constant pressure [9].

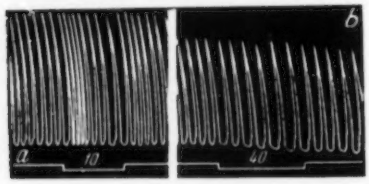

Fig. 2. Experiment on the sheat-fish. Reflex changes in the heart rate by increasing the pressure in the third branchial vessel to 10 (a) and to 40 (b) cm of water.

It appeared of great interest to determine the character of the reflex effects on the vessels in response to stimulation of the receptors of the reflexogenic zone under study. Experiments showed that during distension of the branchial vessels considerable changes could be observed in the volume of perfusate flowing through the intestinal vessels. The vasomotor reflexes were expressed both in the form of dilatation and constriction of the vessels. Fig. 3 illustrates the vasoconstrictive effect caused by an increase of pressure in the branchial vessel, accompanied by a slight slowing of the cardiac activity. Attempts to establish a relationship between the strength of distension of the vessel and the character of the vasomotor response gave less definite results than did the experiments on the heart, but in some cases it was possible to show that the pressor response arose at a lesser degree of distension than the depressor response. It was only comparatively rarely, however, that such a relationship could be observed in a clear form.

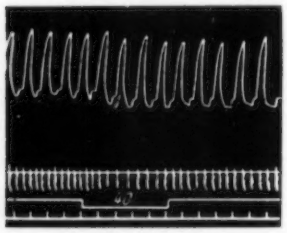

Fig. 3. Experiment on the sheat-fish. Reflex constriction of the intestinal vessels and inhibition of the rhythm of the heart by increasing the pressure in the first branchial vessel to 40 cm of water. Significance of the curves (from above down): mechanogram of the heart, recording of the fall of drops of perfusate flowing through the intestinal vessels, stimulation marker, time marker (5 seconds).

During simultaneous recording of the action of the heart and the vascular reactions it was not possible to show connections between a particular reflex effect on the heart and a particular vascular reaction. For example, inhibition of the rhythm of the heart could be accompanied by either constriction or dilatation of the intestinal vessels.

An increase of pressure in the branchial vessels also had a reflex influence on respiration (movement of the gill coverings). Under these circumstances the most varied changes could be observed: quickening or slowing and increase or decrease of the amplitude of the respiratory movements.

It may be concluded from the experiments described that the branchial vessels of fishes contain receptors, capable of responding to changes in pressure, and leading to reflex effects on the activity of the heart and vessels and on the respiratory movements. An important factor determining the character of the reflex response from this reflexogenic zone is the strength of stimulation of the receptors. This is shown particularly clearly by a study of the reflex responses of the heart: strong stimulation causes inhibition of the rhythm and weak stimulation leads to a quickening of the heart rate. This fact is important not only in describing the function of the reflexogenic zone of the branchial vessels in fishes, but also in

understanding the mechanism of action of the vagus nerve on the heart. The peculiar innervation of the heart in fishes, and also the absence of an inhibitory tonus of the vagus nerves suggest that both inhibitory and stimulatory effects are brought about by means of an active influence of the vagus nerve on the heart, and thus contradict the notion of a specifically inhibitory function of the vagus.

SUMMARY

The author studied the relation between the stimulation strength of the pressor receptors located in the gill blood vessels of fish and the character of the reflex responses of the heart, peripheral blood vessels, and respiration in them. It was demonstrated that with mildly stretched gill blood vessels the rhythm of the cardiac contractions increases, while with vigorous stretching it decreases. Since in fish there is heart tone along the vagus nerves, and the cardiac sympathetic innervation is absent, the reflex intensification of the heart rhythm evidently is caused by the active impulse action of the vagus nerve.

Gill vessel stretching of variable intensity may also cause constriction and dilatation of the intestinal vessels and various changes in respiratory motions.

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THE ROLE OF QUANTITATIVE AND QUALITATIVE FACTORS IN THE FORMATION OF REFLEX VASOMOTOR REACTION TYPES

Yu. I. Rampan

From the Institute of the Experimental Pathology and Therapy of Cancer (Director – Corresponding Member AMN SSSR Prof. N. N. Blokhin) of the AMN SSSR, Moscow

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There are two points of view regarding the formation of quality in the reflex reactions of the blood pressure. Several workers [1, 7, 2, 3, 4, 16] consider that the quality of vasomotor reactions is determined by a quantitative factor, in other words by the intensity of the flow of afferent impulses to the vasomotor center. Under these circumstances they stress the importance of the number of nerve elements (afferent fibers, neurones) involved in the reaction [8, 9, 3]. According to the statements of the supporters of this point of view, the vasomotor afferent fibers, like the corresponding center, are functionally homogenous.

According to the other point of view the nature of vasomotor reflex reactions depends on a qualitative factor, on the specific organization of the elements of the reflex arc. There are reports of a connection between the quality of a reflex vasomotor reaction with both stimulation of the receptors of definite reflexogenic zones and with excitation of particular afferent conductors, and with the possible presence in the central nervous system of narrowly specialized formations affecting the blood pressure in a particular manner [5, 6, 15, 19, 10 and others].

In the present research we studied the role of quantitative and qualitative factors in the formation of vasomotor reflex reactions of pressor or depressor type.

EXPERIMENTAL METHOD

The work was done on dogs, cats and rabbits. The experiments on dogs were carried out under morphine-urethane anesthesia (1-2 cm^3 of 1% morphine and 1 g/kg of urethane), and those on cats and rabbits under urethane anesthesia (1 g/kg). We investigated the reflex vasomotor reactions elicited by stimulation of the nerve trunks separated from the vagus nerve in the neck. The level of the blood pressure in the right carotid artery was measured with a mercury manometer. The respiration was recorded simultaneously by means of a Marey's capsule. The central ends of the separated nerve trunks were stimulated with an induction current of varying strength (current voltage in the primary coil 4.8 v). The frequency of stimulation in the experiments was constant. In all the experiments the vagus and aortic nerves on the opposite side were divided in order to exclude the effect of reflex changes in cardiac activity.

EXPERIMENTAL RESULTS

As a result of the separation of the nerve pathways of the vagus system (16 experiments on dogs, 12 on rabbits and 6 on cats) nerve conductors were isolated from the central ends of which either purely depressor or pressor reactions, sometimes also connected with changes in respiration, were observed in response to various strengths of stimulation with the induction current. In the latter case the type of the vasomotor reactions was not determined by the accompanying changes in respiration; both pressor and depressor reactions took place on

a background of unaltered respiration, and also when respiration was stimulated or depressed. The quality of these vasomotor effects was not affected by changes in the strength of stimulation of the nerve conductors, and hence the presence of narrowly specialized vasomotor afferent pathways in the vagus nerve system was postulated. From the point of view of the "quantitative" hypothesis, however, the varying nature of the reactions of

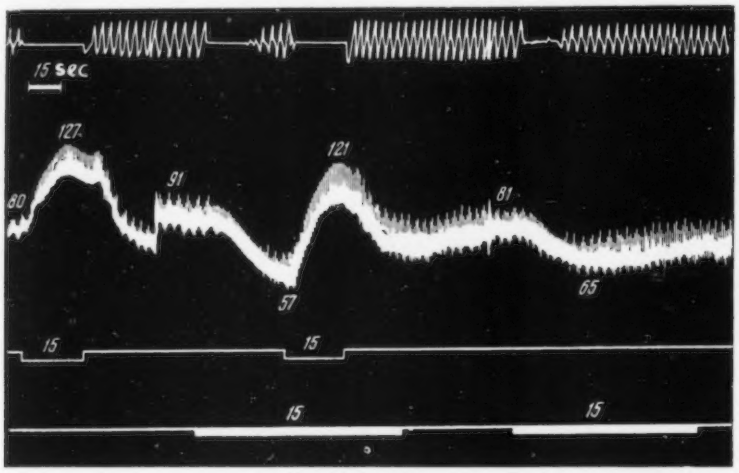

Fig. 1. Changes in the reflex vasomotor reactions in a dog (weight 6.5 kg). Anesthesia: 1 cm^3 1% morphine and 1.4 g/kg urethane. Significance of the curves (from above down): respiration, arterial pressure, stimulation marker. The figures above the stimulation marker show the strength of stimulation in cm (distance between the coils of the induction apparatus). Stimulation of part of the left vagosympathetic trunk, increasing the arterial pressure (upper marker) on a background of the depressor influence from the conductor separated from the right vagosympathetic trunk (lower marker). A weakening of the depressor effect is observed.

the blood pressure observed in response to stimulation of the conductors indicated could only be explained by a difference in the number of vasomotor fibers in these nerves. In order to test this assumption, in subsequent experiments we stimulated the functionally "different" and "similar" vasomotor nerve conductors which we had found jointly, one on the background of the other. For this purpose we used two independent circuits with separate sources of current (7 experiments on dogs, rabbits and cats — 42 combined stimuli).

In response to combined stimulation of the pressor and depressor nerve conductors, weakening of the changes in blood pressure took place in all the species of experimental animals (Figs. 1 and 2). Complete disappearance of the reaction was observed in those cases when the pressor and depressor conductors were stimulated simultaneously in strengths which separately caused equal and opposite effects (Fig. 3). The combined stimulation of two pressor or depressor conductors in the same experiments enhanced the corresponding pressor or depressor effect.

It follows from these findings that an increase in the number of vasomotor afferent fibers involved in the reaction during combined stimulation of conductors with opposite effects on the blood pressure was functionally not equivalent to the same increase in the number of exicted vasomotor fibers during the combined stimulation of "pressor" or "depressor" afferent pathways alone.

These results are evidence against the explanation of the formation of the quality of reflex vasomotor reactions by means of a quantitative factor alone, i.e., by a change in the number of excited neurones in a functionally homogenoeous vasomotor center or by a variation in the strength of the afferent impulses reaching it.

Furthermore, in response to stimulation of mixed vasomotor nerve conductors with varying strengths of current in dogs, the action of the smaller strength was to cause depressor reactions in the animal; stronger currents led to pressor reactions. In rabbits, on the other hand, weaker currents led to pressor reactions and stronger currents to depressor.

These findings are also difficult to explain from the point of view of a definite role of a quantitative factor in the formation of the type of vasomotor reflex reaction, since an increase in the number of excited vasomotor fibers and in the intensity of the afferent impulses regularly led to replacement, in one case (rabbits) of pressor reactions by depressor, and in another (dogs), on the other hand, of depressor reactions by pressor. Such a difference in the relationahip between the type of the vasomotor reactions and the strength of stimulation could evidently be explained by a difference in the quantitative distribution of the pressor and depressor fibers in the vagus nerve system of these species of animals.

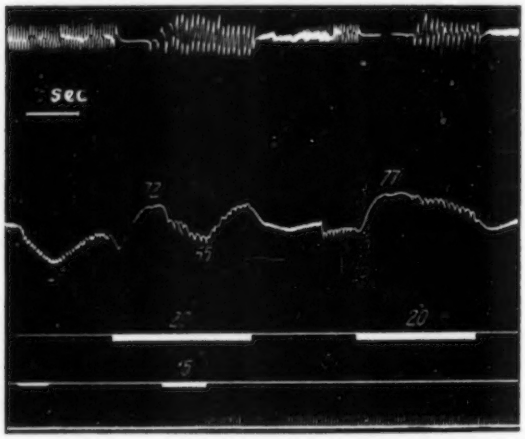

Fig. 2. Changes in the reflex vasomotor reactions in a rabbit (weight 3.5 kg). Left side. Urethane anesthesia 1 g/kg. Significance of the curves (from above down): respiration, arterial pressure, stimulation marker. Stimulation of the depressor nerve (lower marker) on a background of the pressor influence from the vagus nerve (upper marker). A weakening of the pressor effect is seen.

We postulated that the qualitatively different vasomotor nerve conductors which we studied had the same afferent nature, since the central ends of the nerve conductors forming a part of the vagus nerve system were stimulated. It is true that in the experiments on dogs the possibility was not excluded that ascending efferent fibers of sympathetic origin were also stimulated, since in dogs the vagus and sympathetic nerves pass within the same membrane and are difficult to distinguish from each other. Against the sympathetic nature of the vasomotor fibers which we studied during combined stimulation, there was the evidence of our experimental findings that narrowly specialized pressor and depressor pathways were found not only in dogs but also in rabbits, when the central ends of the vagus and aortic nerves in these animals were stimulated. Under these circumstances, in view of the anatomical isolation of the sympathetic fibers in the cervical region, the conditions were not present

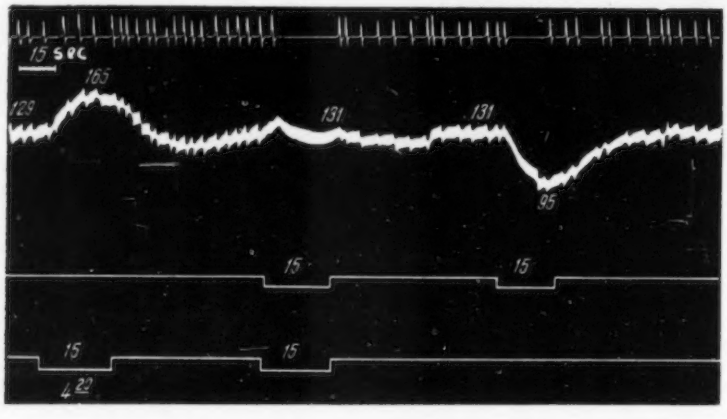

Fig. 3. Changes in the reflex vasomotor reactions in a cat (weight 4.6 kg). Left side. Urethane anesthesia 1.4 g/kg. Significance of the curves (from above down): respiration, arterial pressure, stimulation marker. Mutual weakening of pressor and depressor influences during simultaneous combined stimulation of the vagus (upper marker) and aortic (lower marker) nerves.

for the effecting of sympathetic influences on the blood pressure. That the vasomotor nerve pathways which we studied were not sympathetic was also shown by experiments on cats, in which multiple stimulation of the cervical sympathetic nerve, alone or in combination with the vagus nerve, had no effect on the level of the blood pressure. The absence of an influence of the cervical sympathetic nerve on the blood pressure was also suggested by other workers' results [5, 6, 20, 21, 14]. It may thus be assumed that the qualitatively different vasomotor nerve conductors which we studied (especially in experiments on dogs) were afferent by nature.

So far as the debatable question of the presence of specific reflex reactions of the blood pressure, independent of the number of nerve fibers brought into action, is concerned, it seems to us that our experiments provide new confirmation of the existence of such specific reactions.

Narrowly specialized reflex reactions of a rise and fall in the blood pressure were observed by us in conditions where the influence of receptors was excluded (the central ends of the nerves were stimulated). This showed that the specificity of these reactions was not due to the organization of the receptors. On the other hand, we consider that the quality of reflex changes in the blood pressure is likewise not determined by the structure of the afferent conductors themselves. It follows, for instance, from comparison of the results of our experiments with the action of an induction current on the afferent pathways of the vagus nerve system with the data obtained by a number of workers [19, 10, 17, 18] by direct stimulation of various areas of the medulla oblongata and the hypothalamus, that in both cases the results that took place were the same. This coincidence of the results of two different forms of experiments suggests that the decisive factor in determining the quality of a reflex change in the blood pressure is not the structure of the afferent conductors themselves but the structure of the central formations with which these conductors are connected. There are reports in the literature of the presence of afferent pathways in the vagus nerve system, leading not only to the medulla, but also to the supramedullary nerve formations [11, 12, 13].

On the basis of the foregoing it may be postulated that receptors, excited by adequate stimuli, selectively involve in the reaction one or other afferent nerve pathway of the vagus nerve system. Nerve impulses from this afferent pathway pass to particular narrowly specialized functional nerve formations; the distinctive features of the organization of these formations determine the quality of the reflex vasomotor reaction.

SUMMARY

In dogs, rabbits and cats there are strictly specialized vasomotor afferent pathways connected with the rise or drop of the blood pressure in the vagus nervus system. The quality of the reflex reactions from these pathways does not depend on the number of the nerve fibers excited and the intensity of the afferent impulsation reaching the vasomotor center. The quality of the reflex reactions in stimulating these afferent pathways is evidently determined not by the structure of the conductors proper, but by the features peculiar to the organization of those central formations with which the latter are connected.

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THE TARGET OF THE CHEMICAL STIMULUS DURING PERFUSION OF THE BLOOD VESSELS OF LYMPHATIC GLANDS

Yu. M. Zaretskaya (Moscow)

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The method of perfusion of an isolated organ through its blood vascular system, widely used in the study of chemoreceptors, gives no idea of the part played by the tissue receptors themselves of the perfused organ in the reactions. In other words, it remains unexplained whether the reflex changes in the arterial pressure and respiration observed during the passage of a chemical stimulus through an organ are caused by the action of the stimulus on the tissue receptors or only on the receptors of the blood vessels.

This problem, which was the subject of lively discussion a few years ago, still remains unsolved.

The possibility of interaction between the chemical stimuli usually used – nicotine, acetylcholine, potassium chloride, acetic acid and so on – and the tissue receptors was not in doubt. It remained uncertain, however, whether these substances could, by the use of this method, penetrate through the walls of the blood vessels and escape into the surrounding tissue.

In the present research an attempt was made to elucidate this problem.

EXPERIMENTAL METHOD

As an object for study we chose the chemoreceptor field of the lymphatic glands situated in a large cluster at the root of the mesentery of the small intestine, the so-called Aselli's pancreas, the anatomical features of which facilitated the carrying out of the work (Fig. 1).

The neurovascular bundle passing to Aselli's pancreas contains the mesenteric artery, mesenteric vein and mesenteric plexus. Directly beneath it, in the soft tissues, is situated the great efferent lymphatic duct, collecting the lymph from the whole of the abdominal cavity. There are no other lymphatic glands along its course to the thoracic duct.

As an analog of the stimulating substance, in our experiments we used the dye fluorescein $C_{20}H_{12}O_5$ (molecular weight 332), the molecule of which is larger than the molecules of the chemical stimuli usually used – nicotine, acetylcholine, etc.

The experiment was performed as follows. In a cat, under urethane anesthesia, through a laparotomy incision the mesentery was extracted and Aselli's pancreas isolated from the surrounding tissues, leaving only a bridge of tissue including the efferent lymphatic duct and the neurovascular bundle. The artery and vein were carefully dissected, ligated and divided. The lymphatic vessel was not divided. As a result of these manipulations Aselli's pancreas preserved its connection with the body only by means of the efferent lymphatic duct.

Ringer-Locke's fluid was perfused through the artery and emerged freely from the vein.

Into the perfusate was injected 1 ml of a 5% solution of fluorescein. After 10, 30 and 60 minutes, samples of blood were taken from the common carotid artery, into which a cannula had previously been inserted, the plasma was separated and examined in blue light from a lamp.

EXPERIMENTAL RESULTS

In all the samples the presence of fluorescein was detected by its characteristic greenish luminescence. It was found in greatest amount in the blood samples taken 30 minutes after injection of the dye.

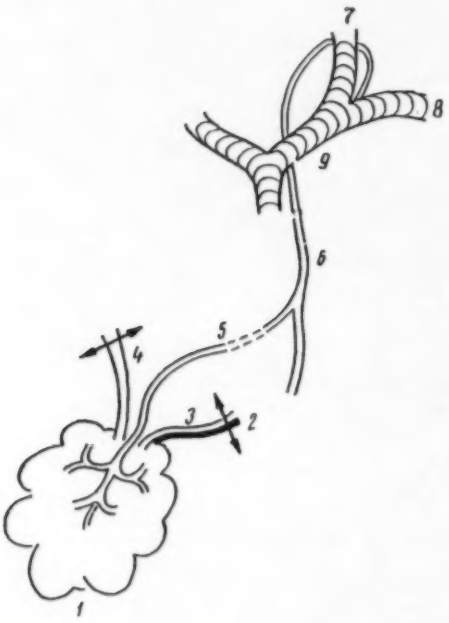

Fig. 1. Anatomical relations in the region of Aselli's pancreas (diagrammatic). 1) Lymphatic glands; 2) mesenteric plexus; 3) mesenteric artery; 4) mesenteric vein; 5) efferent lymphatic vessel; 6) thoracic duct; 7) jugular vein; 8) subclavian vein (left); 9) innominate vein (left) the site of division.

Hence fluorescein injected into an organ isolated from the general blood circulation was found in the blood stream. It is clear that the dye must have entered the blood only through the efferent lymphatic duct, which means that the fluorescein had penetrated from the blood stream into the tissues of the lymphatic glands, thence through the efferent lymphatic duct and the thoracic duct into the general circulation of the blood. We obtained completely identical results in 8 experiments.

Since the penetration of a substance through a vessel wall depends mainly on the size of its molecule, it may be expected that substances having a smaller molecule, such as nicotine, acetylcholine, potassium chloride, acetic acid and so on, during perfusion of an isolated organ by the usual method, will also penetrate into the tissue of the organ examined, where they will interact with the tissue receptors.

We were able to show penetration of fluorescein through the vessel wall by the example of the lymphatic glands, but it must be assumed that this pehnomenon may also take place in other organs (spleen, intestine and so on). It is difficult, however, to perform an analogous experiment on these objects by virtue of the fact that the lymphatic vessels leading from them pass through regional lymphatic glands which hold up the fluorescein.

SUMMARY

With the isolated perfusion of the blood vessels supplying the lymph nodes the fluorescein, as shown experimentally, penetrates through the vascular walls into the tissue of the former.

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CHANGES IN THE GAS EXCHANGE DURING EFFORT IN PATIENTS AFTER OPERATIONS ON THE LUNG

R. S. Vinitskaya

From the A. V. Vishnevskii Institute of Surgery (Director — Active Member AMN SSSR A. A. Vishnevskii, Scientific Director — Active Member AMN SSSR P. K. Anokhin) of the AMN SSSR, Moscow

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Certain pathological processes affecting the lung tissue or surgical operations on the lung interfere with external respiration. Under these circumstances the bodily functions are reorganized in accordance with the necessity of delivering oxygen to the tissues in order to maintain oxidative processes at the required level. In chronic pathological conditions of the lung (carcinoma, bronchiectasis) it is difficult to detect before operation the compensatory mechanisms which are gradually brought into play. Nevertheless the problem of the ways of compensation of the disorders of gas exchange in chronic pulmonary diseases, and also after major interference with the respiratory apparatus associated with operations on the lungs, is one of theoretical and practical interest.

P. K. Anokhin considers that the ordinary mechanisms of maintenance of the stability of the bodily functions in normal conditions have much in common with the mechanisms of compensation of these functions when disturbed [1].

One of the conditions causing reorganization of several bodily functions is physical effort. Much research has been done to study the changes in the bodily functions of healthy persons during effort in the course of athletic training, manual work and so on [5, 7, 11, 13, etc.]. Some interesting work has been done by Hemingway et al. [14], who investigated the changes in gas exchange with age, using physical effort for this purpose. Physical effort is widely used as a functional test nowadays in the examination of patients with diseases of the heart and lungs, since investigations at rest often fail to show certain defects of the respiratory and vascular systems [12, 15-19].

These authors, however, did not dwell on the special features of the adaptive reactions in different patients, which from our point of view is of definite interest in the problem of elucidating the compensatory mechanisms brought into play as the result of an increasing disability of the respiratory apparatus.

In this connection we examined 44 patients with pulmonary diseases at rest and after physical effort, before and after operation, in order to discover any individual peculiarities of the nervous regulation of respiration and the circulation of the blood in response to a raised level of the oxidative processes.

EXPERIMENTAL METHOD

We used the Douglas-Haldane method of investigation of the gas exchange and, in individual cases, Krogh's method. The examinations were carried out in the morning, on fasting animals, in basal metabolic conditions. Measurements were made of the pulmonary ventilation (in liters per minute), the oxygen demand and the excretion of carbon dioxide (in ml per minute), the percentage utilization of oxygen in the lungs, the respiratory coefficient, and the basal metabolism (in calories and as a percentage of normal). At the same time

the pulse and respiration rate were counted. The patients then performed moderate physical exercise (going up and down steps 30 times in 1½ minutes, or sitting down 20 times in 1½ -2 minutes). After the exercise the gas exchange was again investigated in the course of 7-8 minutes (see table).

Details of the Character of the Disease and the Number of Patients Examined

Character of the disease	Total number of patients investigated	Number of patients examined before and after operation
Carcinoma of the lung	22	4
Bronchiectasis	15	3
Benign tumors, cysts, hydatid disease, etc.	7	5
Total	44	12

EXPERIMENTAL RESULTS

As we know, the increased oxygen demand of the tissues during and after effort is brought about by several interconnected functions taking part in the transport of oxygen. Under these circumstances increases take place in the pulmonary ventilation, the minute volume of the heart, the arteriovenous oxygen difference of the blood and so on.

In response to brief physical effort the pulmonary ventilation in all our patients examined increased 1½-2 times, and in the course of 5-6 minutes after the exercise it decreased to approximately the initial value. The oxygen demand (in ml per minute) was 2-3 times greater than the original before the physical effort (measurements were made in individual cases) and twice as great as the initial value in the first 3 minutes of the recovery period, gradually decreasing with rest. The percentage utilization of oxygen in the lings during brief physical effort increased by comparison with the resting state at the beginning of the recovery period and sometimes fell below the initial value at the end of this period, when the oxygen demand did not exceed its initial value. Only in a few cases was the percentage utilization of oxygen below the initial value in the first minutes of the recovery period.

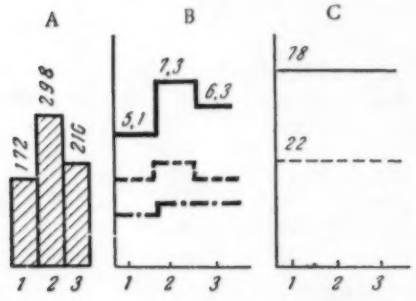

Fig. 1. Changes in the gas exchange, pulse and respiration after physical effort in the patient K., aged 40 years. Diagnosis: carcinoma of the left lung. The pulse and respiration rate were unchanged after the exercise. 1) before exercise, at rest; 2) during the 1st-3rd minutes after exercise; 3) during the 4th-7th minutes after exercise. A) Oxygen demand in ml per minute; B) pulmonary ventilation in liters per minute; percentage utilization of oxygen in the lungs - - - - ; percentage of expired carbon dioxide -.-.-.-. ; C) pulse rate ——— and respiration rate - - - -.

On the basis of an analysis of the results obtained, it was found that the performance of physical exercises, which in all patients doubled the oxygen demand, was achieved by various forms of nervous regulation of respiration and the circulation of the blood. Four main types of reaction of respiration and the blood circulation to brief effort could be noted.

1). In 12 cases an increased delivery of oxygen to the tissues after effort took place as the result of deepening of the respiration and increased utilization of oxygen, as might be assumed, on account of an increase in the stroke volume of the heart. The pulse and respiration rates under these circumstances remained unchanged (Fig. 1).

2). In 17 cases the increased oxygen demand after physical effort was satisfied by an increase in the depth and the rate of respiration and an increase in the percentage utilization of oxygen. The heart rate was unchanged or even became slower, from which an increase in the stroke volume of the heart could be deduced (Fig. 2). The subjects forming the first and second groups had practiced sports in the past.

3). In 8 patients there was a considerable rise in the heart rate after effort, whereas the respiration rate remained unchanged or became slower. The increased oxygen demand was met by a deepening of the breathing and an increase in the minute volume of the heart, in this case mainly on account of an increased rate of contraction. The percentage utilization of oxygen in the lungs was unchanged.

4). In 19 cases all the reactions which we studied were enhanced: the depth and rate of respiration, the percentage utilization of oxygen and the heart rate were all increased (Fig. 3).

In 12 patients whom we were able to examine before and after operation on the lung, it was found that after operation the leading components of the reaction were brought into play to a greater degree (see Fig. 3, A). For example, in the postoperative period the respiration rate in patient L. increased after effort from 14 to 24 per minute, and before operation from 14 to 18 per minute. It must be pointed out, however, that the character of the changes in respiration and in the action of the heart in response to effort remained just as before the operation in 6 of the 12 patients. It may be postulated that this was in consequence of the absence of change in the stereotype of reactions of the nerve centers controlling respiration and the circulation of the blood. A change in the excitation level of these centers after pneumonectomy or other lung operations led in several cases to a considerable increase in the respiration or heart rates in comparison with the preoperative period and also to an increase in the difference between their rates at rest and after exercise. This change in the level of excitation of the centers could possibly be the reason for the fact that in some patients the character of the reactions of respiration and of the circulation of the blood in response to exercise changed after operation.

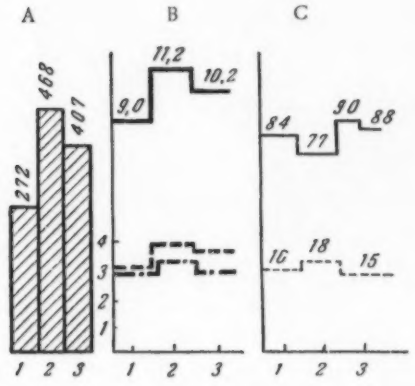

Fig. 2. Changes in the gas exchange, pulse and respiration after physical effort in the patient K., aged 20 years. Diagnosis: bronchiectasis of the right lung. Legend as in Fig. 1. In the first minutes after exercise the pulse rate became slower and the respiration slightly quicker.

In assessing the gas exchange findings particular interest was due to the percentage utilization of oxygen by the lungs; this depends on the minute volume of blood passing through the lungs, on the arteriovenous difference in oxygen concentration, and partly on the oxygen capacity of the blood. In 4 healthy persons whom we examined as controls of the standard exercise usually assigned to the patients, the percentage utilization of oxygen in the lungs was increased considerably after effort (by 1.5-2%). The heart rate under these circumstances was changed to a much lessor degree, which showed an increase in

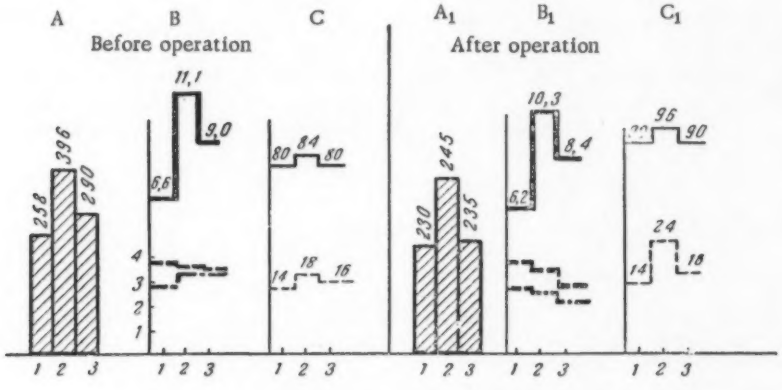

Fig. 3. Changes in the gas exchange, pulse and respiration after physical effort in the patient L., aged 32 years, before and after operation. Diagnosis: hydatid disease of the left lung. The pulse and respiration rates were increased after exercise. The character of the changes as a result of effort was the same before and after operation. A, B, C) Before operation; A_1, B_1, C_1) 3 weeks after removal of a hydatid cyst associated with considerable involvement of lung tissue. Legend as in Fig. 1.

the stroke volume of the heart. It is known from the literature that during training the stroke volume of the heart increases, and that this is probably a more economical mechanism for the transport of the same amount of oxygen than an increase in all the functions.

In more severe disorders of the heart or lungs the opposite was observed.

With insufficiency of the blood flow through the pulmonary circulation a demand was created for an increase in the pulmonary ventilation, the respiration rate was increased and the percentage utilization of oxygen in the lung fell. We observed such reactions in individual patients who had not undergone operation and in some patients in the postoperativeperiod (patient F. and others). Such a state borders on exhaustion of the possibilities of compensation i.e., on decompensation.

From an analysis of the results of the examinations on patients before and after operation, it may be stated that the increased oxygen demand of the body after the performance of physical exertion, just like the compensatory reactions directed toward the delivery of the necessary amount of oxygen to the tissues during a disorder of the respiratory apparatus, may result from different forms of reaction of the cardiovascular and respiratory systems.

Depending on the functional integrity of each of these systems, and also on the system of control developed in the course of the life of each individual subject, the compensatory reactions mainly follow one or other of these courses.

It can be seen from the foregoing that if, in a patient with a disorder of the lung, light physical effort entails a considerable increase in the respiration and heart rate, and under these circumstances the percentage utilization of oxygen in the lungs falls, the compensatory possibilities of such a patient are strained to the limit. The postoperative period will be attended in this patient with great difficulties, and the state of compensation after operation will be established much later on. Compensation in these patients will be unstable.

The four different categories of reactions which we found may be either different types of compensatory shifts in the body in response to the increased gas exchange requirements, or different stages of bringing into play of the individual reactions; they depend on the state of excitation of the nerve centers controlling respiration and the circulation of the blood.

SUMMARY

The author studied the gas exchange, pulse rate and frequency of respiration at rest and following moderate physical exertion in 44 patients suffering from diseases of the lungs.

Both the increased oxygen requirement following physical exertion and compensatory reactions occurring with disturbed respiratory apparatus are effected in diverse groups of individuals through different pathways of nervous control of respiration and circulation. Four types of respiratory and circulatory reactions in response to exertion were revealed. In all patients these reactions were intensified after the operation; in 50% of them modifications of respiration and cardiac activity were of the same type as preoperatively.

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CHANGES IN THE EXCITATION OF THE OPTIC ANALYZER IN MAN BY MICROWAVES

N. I. Matuzov (Leningrad)

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Microwaves are widely used nowadays in industry and medicine. By far the great majority of papers published so far deal with the thermal action of microwaves, and so far as the nonthermal action is concerned, this has not been studied. Some authors [7] assert that, in doses not causing heating of the tissues, microwaves do not in general possess biological activity. The views of these authors were not subsequently confirmed.

It was shown in animal experiments that, with a power flux density of an ultrahigh frequency electromagnetic field (uhf) of 5 milliwatt/cm^2 (the lower limit of thermal activity is accepted to be 10 milliwatt/cm^2), changes occur in dogs not only in the conditioned reflexes [5] but also in the electrocardiogram [6] and the electroencephalogram [1].

In the present research we studied the reactions of the human body, and primarily the central nervous system, to nonthermal doses of microwaves.

Since the optic analyzer in man is the most highly "corticalized" (K. M. Bykov), we judged the character of the effect of these waves on the central nervous system by changes in the functional state of the organ of vision arising as a result of the action of microwaves on man.

EXPERIMENTAL METHOD

In order to ascertain the functional state of the visual analyzer during the action of the microwaves, the functional mobility of the light-perception apparatus of the fundus of the retina and the optic rheobase and chronaxie of the dark-adapted organ of vision were studied.

As an index of the functional mobility of the retina, the area of projection fo the blind spot was determined by means of a scotometer, which we constructed jointly with S. F. Libikh [2].

The optic chronaxie and rheobase were determined by means of an attachement to a sensory chronaximeter, constructed by G. B. Smolyanskii and S. F. Libikh*.

Observations were made on two human subjects aged 23 and 24 years. Both were apparently healthy and had normal vision.

The scotometer, with a white screen and black indicator, was placed before a microwave source with a pulse action. The subjects were seated before the scotometer screen with their back to the source. The illumination of the scotometer screen (70 lux) was constant for all the investigations. In order to determine the area of projection of the blind spot, the subjects were adapted to the brightness of the screen for 5 minutes. After determination of the area of projection of the blind spot, the microwave generator was switched on at $\lambda = 10$ cm. Immediately before the end of 10 minutes of continuous action of the microwaves, the area of projection of the

*Technical improvement cirtificate No. 20 of December 24, 1956.

blind spot was again determined. The area of projection was determined on two further occasions, 10 and 45 minutes after cessation of the action of the microwaves.

In 2 observations on each subject, the area of projection of the blind spot was also studied 120 and 150 minutes after switching off the generator. In view of the fact that the circumstances of the investigation, and in particular the noise of the generator, etc., may affect the opticanalyzer, control observations were made, in which in some cases the source was screened, and in others the subjects wore protective clothing.

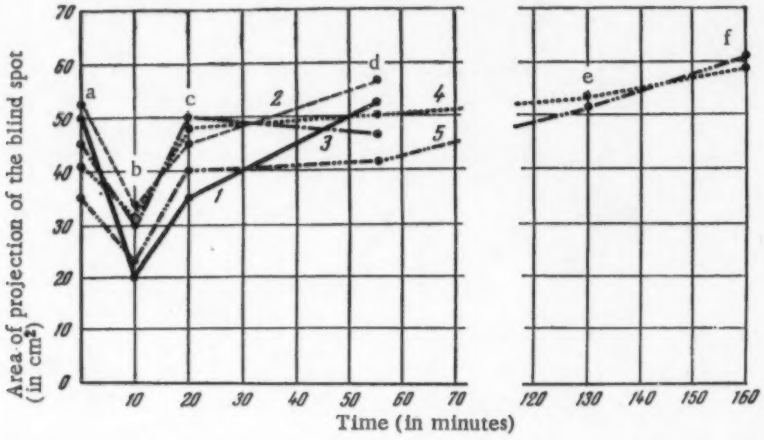

Fig. 1. Changes in the area of projection of the blind spot in the subject N. I. M. during the action of the uhf field. a) Before the action of the microwaves; b) at the end of the action; c) 10 minutes after the end of the action of the microwaves; d) 45 minutes after; e) 2 hours after; f) 2½ hours after; 1, 2, 3, 4, 5) investigation numbers.

This clothing consisted of a cotton combination suit and helmet, and between the outer layer and the lining of the helmet there was a metal net with a fine mesh. Protective spectacles contained the same net instead of glass lenses. The combination suit was worn over the underclothing.

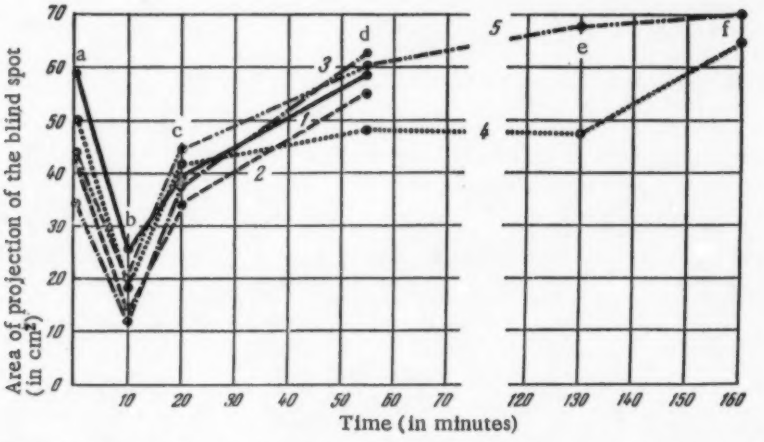

Fig. 2. Changes in the area of projection of the blind spot in the subject I. I. R. during the action of the uhf field. Legend as in Fig. 1.

Determination of the optic rheobase and chronaxie was carried out in a dark room. After adaptation to the dark for 30 minutes a standard exposure to light was given. When 30 minutes had elapsed after the standard exposure to light, the optic rehobase and chronaxie were determined twice at 5-minute intervals. If the results of the two successive determinations were identical, the dark-adaptation was regarded as complete. The uhf generator was then switched on. At the 10th minute of action of the microwaves the determination of the rheobase and chronaxie was repeated, after which the microwave source was switched off.

In the period after the action of the microwaves, the optic rheobase and chronaxie were determined twice: 10 and 15 minutes after switching off the generator. The investigations were repeated every 7-14 days in the period between 2 P.M. and 6 P.M.

The power flux density of the uhf field was 1 milliwatt/cm^2. From data published in the foreign literature the permissible limit of intensity for microwaves of λ=10 cm in industrial conditions is 1 milliwatt/cm^2.

Changes in the Value of the Optic Rheobase and Chronaxie during the Action of Microwaves

Subjects tested	Time	First investigation		Second investigation	
		rheobase (relative figure)	chronaxie (sigma)	rheobase (relative figure)	chronaxie (sigma)
I. I. R.	Before the action of the microwaves	10.4	32	12	30
	At the end of the action of the microwaves	6.2	26	6	27
	10 minutes after the end of the action of the microwaves	10.6	33	11.6	38
	15 minutes after the end of the action of the microwaves	10.2	36	12.4	35
N. I. M.	Before the action of the microwaves	10	38	11	45
	At the end of the action of the microwaves	6.4	26	8	37
	10 minutes after the end of the action of the microwaves	8	30	8.6	25
	15 minutes after the end of the action of the microwaves	15.6	38	13.4	44

EXPERIMENTAL RESULTS

In all, 22 photometric observations were carried out, 10 of which were during the action of microwaves and 12 controls (in 6 the source was screened and in 6 protective clothing was worn). In this way 97 determinations were made of the area of projection of the blind spot.

In order to study the character of the effect of the uhf field on the organ of vision, adapted to dark, 2 investigations were carried out on each subject (4 in all). In these investigations the optic rheobase and chronaxie were determined 20 times.

The results of the determination of the area of projection of the blind spot during the action of the microwaves are shown in Figs 1 and 2.

As may be seen from Figs. 1 and 2, the reaction to the microwaves was clear and almost identical in type in every case, and consisted of a decrease in the area of projection of the blind spot. This decrease demonstrated that during the action of a low intensity uhf field for a short period of time, mobilization of the light-sensitive elements of the retina took place.

From 10-30 minutes after the cessation of the action of the microwaves, the area of projection of the blind spot returned to its original level, and then exceeded it. This increase in the area of projection of the blind spot had not come to an end 2-2½ hours after cessation of the uhf action.

The area of projection of the blind spot in man is known to decrease usually toward evening [4]. The figures given illustrate the opposite picture – increase in the area of projection of the blind spot in the period of action, i.e., toward evening, above the level estimated in the middle of the day.

It is quite possible that this excessive increase in the area of projection of the blind spot is a follow-up reaction, inductive in nature. The results of the determination of the area of projection of the blind spot before application of the microwaves show that the initial value of the area of projection in the subject N. I. M. diminished from investigation to investigation, thereby indicating the presence of a training effect. In the subject I. I. R., the largest area of the blind spot was the same as in the first investigation. We also observed the same effects in another investigation carried out jointly with S. F. Libikh and G. M. Smolyanskii [3].

The course of the curves in Figs. 1 and 2 also shows that neither signs of cumulation nor of increased resistance to the action of microwaves from investigation to investigation could be observed. The absence of such signs may be due to the fact that the action of the microwaves was weak and transient, and the intervals between the individual observations were relatively long. In the observations when the subjects were screened or wore protective clothing, it was found that when the circumstances of the investigation (the noise of the apparatus in action, the illumination and so on) remained as before, the area of projection of the blind spot either was unchanged or slightly increased. This finding was in agreement with the results of the action of noise on the area of projection of the blind spot which we had obtained earlier [2]. The values of the optic rheobase and chronaxie (see Table) under the influence of the microwaves were also diminished, but after cessation of their action, they very quickly (after 10-15 minutes) regained their original level. In the subject N. I. M. in the period after action of the microwaves, the rheobase was even increased above the original level, i.e., the same follow-up reaction took place as with scotometry. It may be concluded from the results obtained that the level of excitation of the organ of vision when dark adapted was raised by the action of the microwaves.

Since the intensity of action of the microwaves in our investigations was clearly within the limits of non-thermal doses, the alterations found in the optic analyzer may be associated with the specific action of these waves. The nature of this action has not yet been determined.

SUMMARY

A considerable decrease of the area of projection of the blind spot, shortening of the adequate optic chronaxia and reduction of the rheobase were observed in 2 persons at the end of a 10-minute period of microwave exposure ($\lambda = 10$ cm with the power flux density of 1 milliwatts/cm^2).

During the aftereffect the above shifts, at first, would come back to their initial level, surpassing it later on in a number of cases.

The intensity of the microwave action excluded the warming of the tissue and, therefore, the changes in the optic analyzer are attributable to the specific effect of the waves.

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THE CONTENT OF SULFHYDRIL GROUPS IN THE BLOOD SERUM IN EXPERIMENTAL RENAL HYPERTENSION AND THE REDUCTION OF HYPERTENSION BY PROLONGED IRON ADMINISTRATION

L. S. Rozanova

From the Department of the Physiology of Animals (Head — Corresponding Member AMN SSSR Prof. Kh. S. Koshtoyants) of the M. V. Lomonosov Moscow State University

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We have investigated the problem of the influence of prolonged administration of iron (in the form of ferrous ammonium citrate) on the course of experimental hypertension in rabbits. Under these circumstances experimental confirmation was obtained of Kh. S. Koshtoyants's hypothesis of the possible hypotensive effect of injections of iron as a stimulator of the biosynthesis of ferritin — a specific iron-containing protein which appears in the blood in hypertension at the stage of stabilization of the pressure and lowers the sensitivity of the vessels to adrenalin.

It has been shown [3] that the level of experimental hypertension may be lowered by the prolonged administration of iron, and moreover that the hypotensive effect may be explained by stimulation of the biosynthesis of ferritin and the intensive functional utilization of this substance by the body.

There are isolated, indirect reports in the literature that sulfhydril compounds are concerned in a chain of specific enzymochemical reactions which play a role in the pathogenesis of the hypertensive state. It has been found that the vasodepressor properties of ferritin and the interaction of ferritin with adrenalin depend on the state of the SH-groups of the ferritin [4, 6].

B. N. Manukhin [2] showed that the tissue SH-groups play a definite part in the mechanism of action of sympathetico-adrenal factors.

In view of these findings, and at the suggestion of Kh. S. Koshtoyants, we conducted the present investigation, devoted to an analysis of the content of SH-groups in the blood serum during the development of experimental hypertension, and in the course of the lowering of the blood pressure by our method of prolonged administration of iron.

EXPERIMENTAL METHOD

In the quantitative estimation of the SH-groups in the blood serum we used the method of amperometric titration of Kolthoff and his co-workers [5], as modified by T. M. Turpaev and S. N. Nistratova. A volume of 0.5 ml of serum, obtained immediately before titration, was mixed with 30 ml of 0.9% NaCl and titrated with 0.001N $HgCl_2$. To record the diffused current we used a microammeter with a sensitivity of $1 \cdot 10^{-8}$ a. In successive titrations of the same sample, the error did not exceed 4%.*

Experimental hypertension was induced in rabbits by partial clamping of the renal arteries. Iron was injected intravenously in the form of a solution of ferrous ammonium citrate (in a dose of 1.5 mg iron per kg

*I express my gratitude to S. N. Nistratova for her help in mastering the technique of amperometric titration of SH-groups.

body weight) on the day before the operation to clamp the renal arteries, and daily thereafter throughout the whole period of observation. Blood for analysis was taken from the marginal vein of the ear, 2-3 times before operation as a rule, and then once every 10-15 days throughout the experiment (30-240 days after operation).

Four groups of rabbits were investigated: the 1st group (10 animals) were normal rabbits; the 2nd group (15 animals) were rabbits with experimental renal hypertension (blood pressure 25-35% above the normal level); the 3rd group (12 animals) were rabbits with experimental renal hypertension and receiving iron (the blood pressure in these animals was 20-40% below that of the animals of the 2nd group); and the 4th group (10 animals) were normal rabbits receiving iron.

EXPERIMENTAL RESULTS

From our study of the content of SH-groups in the blood serum of 56 normal rabbits we found that it varied between wide limits – 38-78 μM/100 ml – being on the average 55.8 μM/100 ml. These findings were in agreement with those of Weissman and his co-workers [7], who showed that the content of SH-groups in human blood serum was 48.8-59.0 μM/100 ml.

From repeated analyses carried out over a long period of time (2 months), it followed that the level of SH-groups in the blood serum was a reasonably constant value for any one animal (given the same physiological conditions). In a group of 10 normal rabbits (1st group) the maximum variation was on the average ± 6.8%.

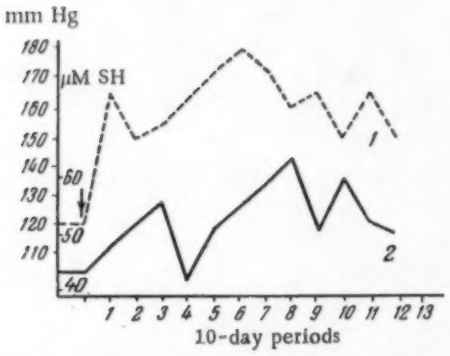

Fig. 1. Changes in the blood pressure (1) and content of sulfhydril groups (2) during the development of experimental hypertension. Rabbit No. 128. ↓ – moment of operation to clamp the renal arteries.

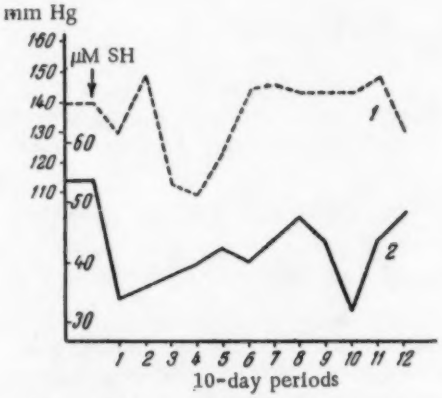

Fig. 2. Changes in the blood pressure (1) and the content of sulfhydril groups (2) during the development of experimental hypertension in association with the prolonged administration of iron. Rabbit No. 141. ↓ – moment of operation of to clamp the renal arteries.

During the development of experimental renal hypertension (2nd group of rabbits; Fig. 1) in 80% of cases an increase was observed in the content of SH-groups which at certain times reached 120-150% of the initial level, i.e., was considerably higher than the normal variations. Sometimes (in 50% of cases) in the first days after operation or, conversely, after 3-4 months, in the stage of stabilization and of a slight fall in the pressure, a decrease took place in the level of the SH-groups to 70-90% of normal; as a rule, however, it was of very brief duration. No clear relationship was found between the level of the blood pressure and the content of SH-groups in the blood serum.

In the 3rd group of animals (rabbits with experimental renal hypertension and receiving iron), in 100% of cases a fall in the content of SH-groups to 60-85% of normal was observed; under these circumstances the level usually remained low throughout the whole period of observation (Fig. 2). In 30% of cases slight rises in the level of the SH-groups were recorded at different times after operation, but these values very soon fell again below their original level. If, therefore, during the development of experimental hypertension there was an obvious tendency for the content of SH-groups to rise, then when the level of the hypertension fell as the result of the prolonged administration of iron, there was a clearly marked fall in the content of SH-groups in the blood serum.

Injections of iron into normal animals (4th group of rabbits) in 80% of cases caused a fall in the content of SH-groups in the blood serum to 80-85% of their initial level, i.e., the effect was shown less clearly than after injection of iron into rabbits with experimental hypertension.

The results obtained led to the conclusion that sulfhydril compounds are one of the links in the complex chain of enzymochemical reactions which play a role in the pathogenesis of the hypertensive state.

B. N. Manukhin [2] showed that the action of the sympathetico-adrenal system depends on the SH-groups of the tissues: during disulfidization the sensitivity to the adrenal factor falls, whereas the presence of substances preventing oxidation of SH-groups leads to stimulation of the action of adrenalin.

Bearing in mind the numerous reports of the role of the sympathetico-adrenal system in the development of hypertension, and also the work cited above on the role of SH-groups in the ferritin — adrenalin system, the role of SH-groups may be represented in the most general terms as follows. Excess of SH-groups in the blood, observed during elevation of the blood pressure, stabilizes adrenalin, increases the sensitivity of the vessels to it, and thereby provides for maintenance of the blood pressure at a high level. On the other hand, a decrease in the content of free SH-groups, caused by a shift in the metabolic processes associated with the intensified biosynthesis of ferritin, leads to intensive oxidation of adrenalin, to lowering of sensitivity to it and, as a result, to some decrease in the level of the hypertension. Indirect confirmation of this hypothesis may be given by the findings of M. Yu. Gaisinskaya [1], who showed that the blood serum has a stabilizing influence on adrenalin, and moreover, that the stabilizing systems become more active in hypertensive disease and in experimental renal hypertension.

The zigzag character of the curve of content of SH-groups during the development of hypertension (2nd and 3rd groups of rabbits; see Figs. 1 and 2) may evidently be explained by the complex interaction between two processes: a pathological, leading to a rise in the pressure and associated with an increase in the content of SH-groups, and a protective, causing a fall in the pressure and accompanied by a lowering of the level of SH-groups in the blood serum. Under these circumstances the pathological process dominates in the animals of the 2nd group, and in the animals of the 3rd group, receiving iron, the protective process is more in evidence. The fact that administration of iron causes a greater reaction in rabbits with hypertension than in normal rabbits shows that the character of the protective reaction in the first case is fairly complex and cannot be reduced to simple stimulation of the biosynthesis of ferritin in the body.

SUMMARY

The authors examined the content of SH-groups in the blood serum of rabbits in development of experimental renal hypertension and in reduction of the latter by chronic iron administration. It was demonstrated that hypertension is accompanied with the rise of the SH-groups content in the blood serum, while in decrease of hypertension by 20-40% as a result of chronic iron administration, the content of the SH-groups becomes lower than its initial value. The data obtained are being considered in connection with the adrenalin and ferritin metabolism in hypertension.

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THE EFFECT OF IONIZING RADIATION ON THE MICROFLORA AND COURSE OF EXPERIMENTAL INFECTED WOUNDS

A. A. Vodyannikova

From the Sverdlovsk Research Institute of Reconstructive Surgery, Traumatology and Orthopedics (Director — Corresponding Member AMN SSSR Prof. F. R. Bogdanov)

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The action of ionizing radiation (x-rays, γ-rays etc.) on the body has been studied in research by V. Ya. Aleksandrov, P. D. Gorizontov, P. N. Kiselev, B. N. Mogil'nitskii and other workers [1, 3, 5, 6].

Ionizing radiation produces lesions in several systems and organs, and interferes with their functions. Among these are the functions responsible for the defense of the body against microorganisms.

We accordingly considered that it would be useful to investigate the microflora and the character of the inflammatory reaction during total irradiation of animals with x-rays.

The study of this problem is of practical as well as theoretical interest. During x-ray therapy and in operations on irradiated tissues it is essential to know what course will be followed by the processes of inflammation and regeneration of the wound in cases where the contamination by microorganisms is only very slight.

EXPERIMENTAL METHOD

Experiments were carried out on 96 white rats and 138 white mice, by L. G. Peretts's method [8], which we used in our previous research [2].

A small incision was made in aseptic conditions in a shaved area of the skin of the dorsal surface of the animal, and by means of a palette-knife the skin, together with the subcutaneous cellular tissue, was separated from the muscles. A known volume of a suspension of microorganisms was introduced from a graduated pipette into the skin pocket thus formed.

For infection of the wound we used strains of Staphylococcus haemolyticus, Bacillus proteus vulgaris and Bacterium pyocyaneum. The largest of experiments was performed with the hemolytic staphylococcus.

As a preliminary step we determined the minimal dose of microorganisms for the experimental animals to cause a local inflammatory reaction with a favorable issue.

The animals were irradiated by means of an RUM-3 apparatus, the total dose for the rats being 500 r and for the mice 240 r (sublethal doses). The animals in group I were first inflicted with the infected wound, and 24 hours afterwards were subjected to total irradiation. The animals in group II were irradiated first, and the infected wound was inflicted 24 hours later. The animals of group III acted as controls, i.e., wounds were inflicted but no irradiation was carried out.

The results of the experiments on white mice were assessed by counting the numbers of dying and surviving animals, by the postmortem findings and by bacteriological cultures from the wound and internal organs, and in the white rats, in addition, on the basis of cytological investigation of wound impressions [4].

In order to study the course of the wound, bacteriological and cytological investigations were made at definite intervals of time (after 24 hours and 3, 7 and 12 days), for which purpose 3 rats in each group were killed at the times indicated.

The microflora was examined by means of subcultures of the wound contents and internal organs in meat-peptone broth and on blood agar, followed by study of the cultural, morphological, plasma-coagulating and hemolytic properties of the microorganisms thus isolated.

EXPERIMENTAL RESULTS

It will be seen from the data in Fig. 1, a that only 10% of the animals in the unirradiated (control) group died. At postmortem, a slight enlargement of the spleen and slight swelling of the intestine were found. The original organism — the hemolytic staphylococcus — was found only in the region of the subcutaneous pocket.

In the first days after x-ray irradiation of white mice with a recently infected wound (Fig. 1, b), the majority of animals died (70%). At postmortem, considerable enlargement of the liver, kidneys and spleen, and edema of the lungs were found.

Inoculation of nutrient media with material from the wound, the heart, liver, spleen and kidneys gave an abundant growth of Staph. haemolyticus with a positive plasma-coagulation reaction and a well-marked zone of hemolysis on blood agar. In films from meat-peptone broth, cocci of various sizes and arrangements, and small Gram-positive and Gram-negative bacilli were seen in addition.

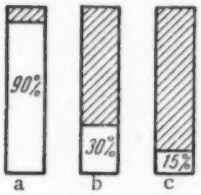

Fig. 1. The effect of total irradiation with x-rays on white mice with infected wounds. a) Control (infection of wound without irradiation); b) infection of wound, followed 24 hours later by irradiation; c) irradiation, followed 24 hours later by infection of the wound.

In the experiments in which mice were irradiated first (Fig. 1, c) the percentage of dying animals was even higher (85%). At postmortem, besides the changes mentioned, hemorrhagic inflammation of the intestine was found. The original organism was isolated from the wound and the internal organs, giving a luxuriant growth on blood agar with a wide zone of hemolysis. Other bacteria were also found, with predominance of bacillary forms.

In the unirradiated white rats, at the site of introduction of the organism causing wound infection, after 24 hours insignificant signs of inflammation were seen, taking the form of the appearance of slight swelling in the region of the subcutaneous pocket and infiltration of the tissues. From the wound contents a culture of the original hemolytic staphylococcus was isolated, with a positive plasma-coagulation reaction (after 6 hours) and a small zone of hemolysis on blood agar. In wound impressions neutrophils were predominant, with active phagocytosis, and only solitary freely situated staphylococci were to be seen.

Three days later the inflammatory focus remained small in size and was encapsulated. Inoculation of nutrient media gave feeble growth of Staph. haemolyticus. After seven days, no signs whatever of inflammation were seen at the site of the incision and introduction of the organisms. Cultures taken from the region of the subcutaneous pocket showed no growth of microorganisms. In wound impressions a small number of phagocytosed neutrophils, basophilic plasma cells and profibroblasts, with solitary plasma cells were seen (Fig. 2, a). After 12 days neither macroscopic nor microscopic signs of inflammation were found. The general condition of the animals in the period of observation was good; cultures taken from their internal organs gave no growth ofthe original organism.

A different picture of inflammation with predominance of destructive processes and generalization of the local suppurative focus was observed in the irradiated animals.

In animals with preliminary infection of the wound and subsequent irradiation, swelling was observed after 24 hours in the region of the subcutaneous pocket; on incision the tissues were edematous and slightly infiltrated with pus. Inoculation of blood agar with material from the wound gave growth of Staph. haemolyticus with a well-marked zone of hemolysis. At postmortem, the internal organs were without visible changes. The original organism — the hemolytic staphylococcus — was isolated from the liver. In wound impressions a large number of destroyed neutrophils and neutrophils with incomplete phagocytosis were seen, and also a considerable number of free staphylococci.

After three days the signs of inflammation were more prominent. At postmortem, slight enlargement of the liver, spleen and lymphatic glands was found. Culture of the wound contents gave growth of the hemolytic staphylococcus with a wide zone of hemolysis on blood agar. The original strain of staphylococcus was isolated from the liver and spleen. In wound impressions degenerated forms of neutrophils were predominant and an abundance of freely arranged staphylococci was observed, together with solitary neutrophils in the act of phagocytosis.

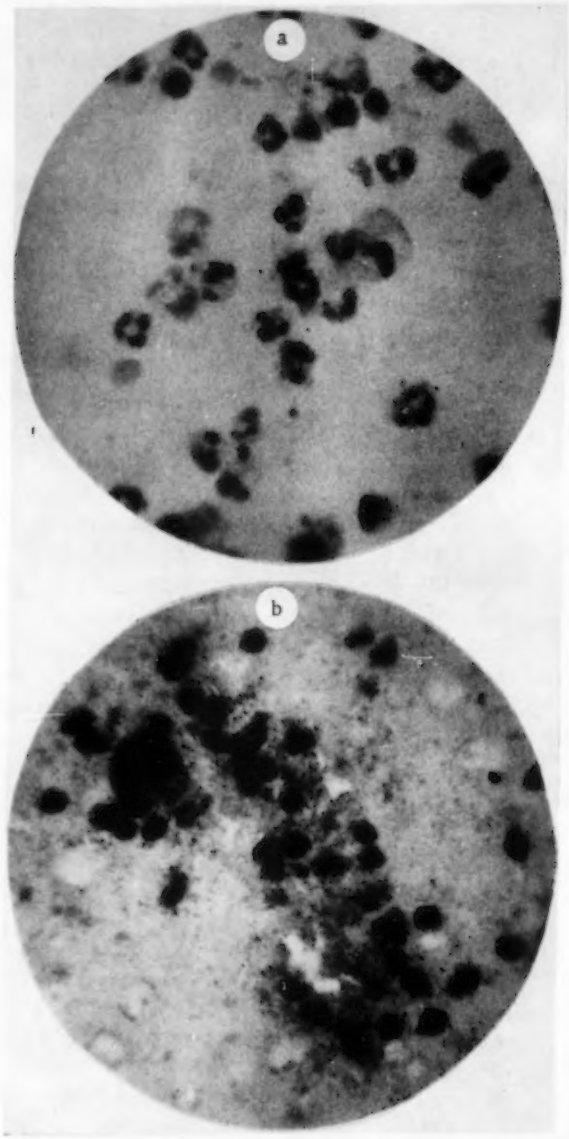

Fig. 2. Impression of the contents of an infected wound in unirradiated (a) and irradiated (b) animals.

After 7 days widespread necrosis of the skin with ulcer formation was found in the region of the subcutaneous pocket. At postmortem, considerable enlargement of the liver and spleen was seen and metastatic foci of suppuration were present in the peritoneal cavity. A hemolytic staphylococcus was isolated from the wound contents which showed a positive plasma-coagulation reaction after 2 hours. Besides the original organism, representatives of the ordinary intestinal microflora were isolated from the liver and spleen.

After 12 days the surviving animals (78%) showed a picture of sluggish inflammation with necrosis of tissues and ulceration at the site of introduction of the microorganisms. At postmortem, the internal organs were edematous; the liver and spleen were enlarged. Necrotic areas were observed on the surface of the liver. A hemolytic staphylococcus with clearly marked pathogenic properties was isolated from the wound. The original organism and intestinal microorganisms were also isolated from the heart, liver and spleen.

On cytological examination of the wound impressions a small number of cells was observed with predominance of degenerative forms of neutrophils; phagocytosis was either incomplete or absent altogether. Besides neutrophils, solitary Unna's cells were found, together with a vast number of freely distributed staphylococci.

The course of the wound process was of an analogous character, although with more pronounced signs of depression of the local reactive processes and with the abundant development of microorganisms not only in the wound but also in the internal organs, was observed in those animals irradiated in the first place.

In the animals of this group, only 24 hours after the moment of infection of the wound, necrotic processes were observed to predominate over inflammatory. Cultures from the wound and from the liver and spleen gave an abundant growth of the hemolytic staphylococcus with clearly marked signs of pathogenicity – a sharp and wide zone of hemolysis on blood agar and a positive plasma-coagulation reaction developing after two hours. In wound impressions destroyed forms of neutrophils predominated and there was a considerable number of freely distributed staphylococci.

On the third day extensive necrosis of the skin with ulcer formation was observed in the region of the subcutaneous pocket. Cultures from the wound and organs, and the cytology of the wound impressions were evidence of the active development of the microflora and of the supression of phagocytosis.

After 7 days the course of the wound process was even more severe, with the development of secondary foci of suppurative inflammation in the peritoneal cavity. In wound impressions degenerated forms of neutrophils and an abundance of freely distributed staphylococci were found (Fig. 2, b). After 12 days the majority of the animals had died with signs of a clearly marked bacteriemia.

In all the irradiated animals, especially those irradiated before infection of the wound, the general condition was found to deteriorate, the animals losing weight and often refusing their food.

Analogous results were obtained in the irradiated animals in which B. pyocyaneus and B. proteus were used as the agent of wound infection.

The investigations thus showed that irradiation with x-rays of animals with local foci of suppuration causes generalization of the process and bacteriemia. This is in full agreement with the reports in the literature of a depression of the barrier functions of the tissues (especially of the lymphatic glands) and a fall in the bactericidal power of the blood in irradiated animals.

In all the experiments the irradiated animals showed a rapid increase in the number of microorganisms whose pathogenic properties were more pronounced than those of the original strain (increase in the plasma-coagulating and hemolytic properties).

During irradiation the inflammatory process was inhibited, whereas in the unirradiated animals a vigorous inflammatory process with active phagocytosis was observed. The phagocytic function of the neutrophils was suppressed in the irradiated animals, which created favorable conditions for the development of an abundant wound microflora.

The experiments showed that the intensity of the process of wound infection also depends on the time of irradiation. For instance, in the animals in which irradiation followed the infliction of an infected wound, it resulted in a less severe course of the wound than in the animals irradiated beforehand.

Presumably the healthy, unirradiated animal, in the first hours, mobilizes all its defensive mechanisms to combat the microorganisms, destroys them at the site of administration and prevents them from penetrating from the wound into the internal organs. Preliminary irradiation suppresses these defensive properties of the tissues, creating conditions in the wound in which proliferation of microorganisms takes place unhindered, so that they invade the blood and internal organs.

SUMMARY

Ionizing radiation affects a number of systems, organs and their functions (among them the functions providing the antimicrobic protection of the body).

Experiments conducted on 96 white rats and 138 white mice demonstrated that x-ray irradiation of the animals with local suppurative focus causes an acute depression of phagocytosis and pronounced increase of the number of microbes in the wound with increase of their pathogenic properties. This promotes the generalization of the process, bacteriemia and causes the death of the animals.

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EXPERIMENTAL OSTEOMYELITIS AND SOME PROBLEMS OF ITS PATHOGENESIS

V. A. Bashinskaya

From the Department of Roentgenology (Head — Corresponding Member AMN SSSR Prof. D. G. Rokhlin) and the Department of Histology (Head — Prof. G. S. Strelin) of the I. P. Pavlov First Leningrad Medical Institute (Director — A. I. Ivanov)

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Ideas of the pathogenesis of osteomyelitis were considerably developed by the research of S. M. Derizhanov [1, 2]. However, neither S. M. Derizhanov nor other workers using his technique in their research, studied certain fundamental problems of the pathogenesis of experimental osteomyelitis in sensitized animals; in particular, the importance of neurovascular disorders in the pathogenesis of this disease was not explained.

D. G. Rokhlin pointed out that in the study of the pathogenesis of osteomyelitis it is essential to take into consideration the important role of prolonged spasm of the vessels. In view of the anatomical and physiological peculiarities of bone, in certain immunobiological conditions this factor may have an essential effect on the development of the disease and may be responsible for its character. The type of sequestra depends on the peculiarities of the bone and on the site of spasm of the vessels.

The aim of the present investigation was to ascertain the importance of neurovascular disorders and, in particular, the role of prolonged spasm of the vessels in the pathogenesis of experimental osteomyelitis. In the work we, therefore, dealt with the following problems: 1) to reproduce experimental osteomyelitis in animals; 2) to make a detailed study, by means of x-ray examination, of the development of the osteomyelitic process and to associate the appearance of its different forms with particular disturbances of the blood supply of the bone; 3) to ascertain the role of prolonged spasm of the vessels in the pathogenesis of experimental osteomyelitis by the use of ganglion-blocking drugs.

EXPERIMENTAL METHOD

Experimental osteomyelitis was induced in rabbits by the method suggested by S. M. Derizhanov. In this series, experiments were performed on 68 animals — males and females weighing from 1800 to 2500 g. Twelve rabbits acted as controls, and 56 were given five preliminary sensitizing injections of normal horse serum, subcutaneously, in the hindlimb, in volumes of 2-3 ml and at intervals of 5 days. From 8 to 10 days after the last subcutaneous injection, 2-4 ml of horse serum was introduced through a drill hole directly into the medullary cavity in the middle part of the tibial diaphysis. This was the assaulting dose of horse serum. The same operation was performed on the control animals, but without preliminary sensitization. From 10 to 20 minutes after introduction of the assaulting dose, 18 of the 56 rabbits died from anaphylactic shock. None of the control animals died. The 38 surviving experimental animals and the 12 control animals were kept under observation for various periods of time.

EXPERIMENTAL RESULTS

Despite the fact that all the rabbits were kept under identical conditions, the experimental animals differed

greatly from the controls in their general condition. The disease was expressed clinically in these animals by an increase in the temperature, slight loss of weight, edema of the injured limb and the formation of an area of infiltration and erythema of the skin at the site of the operation wound. In the blood, the ESR was raised to 20-25 mm in 1 hour, and there was a fall in the hemoglobin concentration and a rise in the leucocyte count to 25,000 per mm^3. The control rabbits were clinically indistinguishable from animals not undergoing operation. Two weeks after introduction of the assaulting dose of serum into the marrow cavity, a systematic series of x-ray films was taken of both hindlimbs of each animal; at first once a week and then twice a month. Under these circumstances we directed special attention to the detailed recording of the pathological changes in the bone and to the course of their development. No osteomyelitis could be detected in any of the control animals. In the experimental animals, on the other hand, one of the forms of osteomyelitis, with an acute, subacute, or chronic course, was observed in all cases.

Analysis of the x-ray films showing the development of the pathological changes indicated that usually on the 20th-21st day, and sometimes earlier, changes appeared both in the soft tissues around the bone and in the periosteum, and also in the cortical substance and the marrow cavity. At this time, on the x-ray films it was possible to observe localized edema of the soft tissues at the level of the drill hole; sometimes the edema was more diffusely spread, affecting the entire limb below the knee. Depending on the character of the developing pathological changes, the edema of the soft tissues lasted for a long time or gradually diminished and completely disappeared.

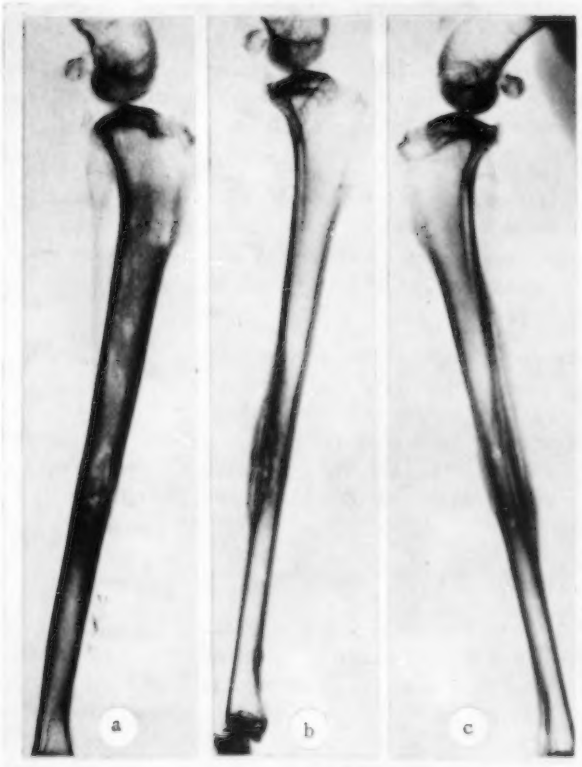

Fig. 1. Osteomyelitic and necrotic changes in the marrow substance in experimental osteomyelitis in rabbits.

On the x-ray films of the 20th-25th day a fine shadow of thickened periosteum appeared in the region of the drill hole, to some extent stripped from the compact bone. Subsequently these periosteal deposits increased in size; the bone was thickened either regularly or irregularly. In contour they were undulating, but smooth; however, in association with the passage of pus (in the presence of a sinus) the contours became, as it were, eroded.

The x-ray films at the beginning of the 4th week, or sometimes later, showed destruction of bone tissue, which took place not only in the region of the operation wound but also far from its limits. Sometimes the pathological process spread from the proximal to the distal metaphysis. Destruction was shown both by osteolysis and by the appearance of necrotic (from their x-ray appearance) areas of different sizes and shapes. At the end of the second month progression of the pathological changes was usually observed, and at the 4th month the lesion reached the peak of its development. The x-ray films at this period showed clearly the formation of sequestra, cavities and sequestral capsules, delineating the affected focus from unchanged bone. In other cases the acute inflammatory changes began to quiesce, and the entire process, becoming localized, acquired a chronic character. In addition to the appearance of cavities and sequestra, irregular sclerosis of bone was seen to develop both periosteally and endosteally.

In our experiments there were cases in which the changes were localized mainly in the periosteum, and the developing process terminated at the end of the 4th-5th month (the most favorable course). From the very beginning edema of the soft tissues was seen, and after 3 weeks stripping of the periosteum was seen in a localized

area of the tibial diaphysis at the level of the drill hole on the x-ray films. Two weeks later this process had spread considerably and the deposits under the periosteum had undergone partial assimilation. Occasionally the process terminated in complete assimilation of the subperiosteal deposits. In such cases the changes did not extend into the depth of the bone.

In certain rabbits the inflammatory process was localized, at a certain stage of development, to the subperiosteal layer of compact substance of the bone. It was very characteristic that, although it spread over a considerable part of the surface, it did not penetrate deeply and did not affect the supraspongiosal layer nor the marrow cavity. At later stages in some rabbits we observed sequestrum formation, the separation being confined to superficial areas of the subperiosteal layer of the compact substance of varying extent. The form of osteomyelitis described must be due to disturbance of the circulation of the blood in a restricted area of the periosteal network, since the subperiosteal layer of the compact substance is supplied by this system of vessels. According to D. G. Rokhlin [6] the supraspongiosal layer of compact substance and the medullary cavities are supplied with blood from the diaphyseal network.

In Fig. 1a, are shown multiple osteomyelitic and necrotic changes in the medullary cavity in the presence of destruction of only the supraspongiosal layer of the cortical substance, a feeble periosteal reaction and absence of any well-marked edema of the soft tissues.

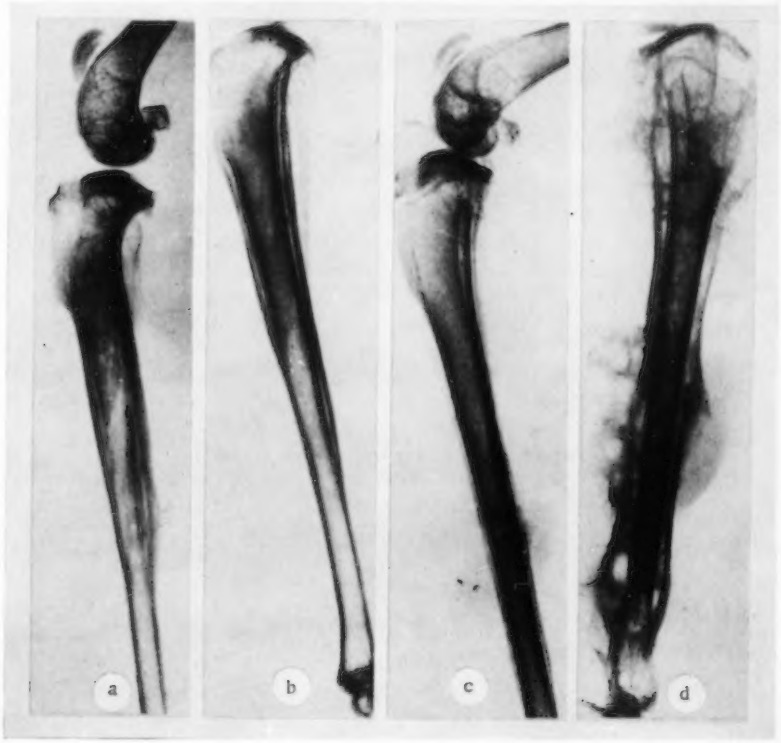

Fig. 2. Total central necrosis in experimental osteomyelitis in rabbits.

Conversion of the subperiosteal and supraspongiosal layers into long, rod-like sequestra at the level of the drill hole is readily seen in Fig. 1, b; osteolytic changes are also seen in a small area of the substantia spongiosa. The sequestra are covered externally by highly assimilated periosteal deposits.

Necrosis of the subperiosteal layer of the compact substance and partial osteolysis of the adjacent supraspongiosal layer, in the presence of intensive periosteal deposits are shown in Fig. 1, c. In this rabbit a pathological fracture occurred in the affected tibia at the junction of the middle and distal thirds.

A case of total central necrosis with pathological fractures in one of the experimental rabbits is demonstrated in Fig. 2, a. Well-marked assimilated periosteal deposits are observed on the posterior surface of the tibia. Total central necrosis, arising in other rabbits, is illustrated in Fig. 2, b. No clearly expressed periosteal changes are seen in the x-ray films in these cases. The development of pathological changes, as shown on later film, must be due to disturbance of the circulation of blood in the branches of the main diaphyseal artery supplying the marrow cavity and the supraspongiosal layer.

It was possible, experimentally, to obtain total necrosis of the bone, i.e., necrosis of the whole thickness of the bone. This was possible only by cessation of the blood supply from the diaphyseal and periosteal networks of vessles.

Fig. 2, c illustrates total osteomyelitis of the proximal $^2/_3$ of the tibial diaphysis. The whole extent of the area undergoing necrosis is surrounded by periosteal deposits. In Fig. 2, d is shown a case of severe chronic osteomyelitis with total necrosis and sequestration of the whole diaphysis of the tibia (from the proximal to the distal metaphysis). Pathological fractures are seen, together with extremely extensive periosteal deposits, in places doubling the thickness of the bone. Well shown on the x-ray films are multiple sinuses and also marked edema of the soft tissues.

Fig. 3. Osteolysis of the substantia spongiosa (a) before and (b) after treatment of experimental osteomyelitis.

On the basis of the x-ray examinations we distinguished several different types of sequestrum formation in experimental osteomyelitis. These types or forms were mainly identified by considering the localization and duration of the process. The presence of different forms of osteomyelitis may be explained by disturbance of the blood supply—by prolonged spasm of the vessels in either the periosteal network or in the diaphyseal artery and its branches or in both.

Regarding the development of osteomyelitis in sensitized animals as the result of spasm of the vessels (D. G. Rokhlin), we attempted to discover, by the use of drugs, the importance of spasm of the vessels supplying the bone in the pathogenesis of the disease. In the experiments which we set up (on 70 rabbits), ganglion-blocking (spasmolytic) substances — hexamethonium, trasentine — were injected in therapeutic doses calculated per kg body weight, immediately or 1-2 hours after the assaulting injection and thereafter 2-3 times daily for 6-20 days. Control rabbits received no injections of ganglion blocking drugs. The development of osteomyelitis in the rabbits of the control series and the animals receiving ganglion-blocking drugs was systematically followed by x-ray examination for 6 months.

As a result of the observations it was found that in the control rabbits, which received no injections of these spasmolytic drugs, osteomyelitis developed in 100% of cases (with one or other type of sequestration). In the rabbits which were injected with hexamethonium or trasentine osteomyelitis failed to develop in 90% of cases.

After obtaining these results, we used ganglion-blocking drugs for therapeutic purposes. We utilized some of the rabbits of the control group in which an osteomyelitic lesion was found. As an illustration we give the following example of hexamethonium treatment of a rabbit which had already developed osteomyelitis. In Fig. 3, a, osteolysis of the substantia spongiosa in the proximal third of the diaphysis of the bone and tiny necrotic areas in the substantia spongiosa may be seen radiographically. As a result of osteoporosis, the compact layer in the proximal metadiaphyseal zone cannot be traced along the posterior surface. Fine, undulating periosteal deposits may be seen over a rather extensive area. Two months after the beginning of treatment with hexamethonium, the bone of this rabbit was normal in structure, which had undergone complete restoration (Fig. 3, b). Similar results were obtained in other animals. These findings support the view put forward by D. G. Rokhlin, that spasm of the vessels is important in the pathogenesis of osteomyelitis. It seems to us that they open up wide prospects for the use of ganglion-blocking drugs in the prophylaxis and clinical treatment of osteomyelitis, used in conjunction with all the measures which are at present employed.

SUMMARY

Several typical forms of experimentally induced osteomyelitis were obtained in 68 rabbits by Derizhanov's method. X-ray examination demonstrated that the pathological process was localized in the periosteum or in various layers of the compact bone substance. Total necrosis was revealed in certain cases. In conformance with the belief that there is a separate blood supply of various layers of the compact substance of long bones a conclusion is drawn that a varying localization of the process is associated with disturbances of the blood supply in certain blood vessels.

A prolonged spasm of the blood vessels plays, apparently, the main role in the disturbance of the blood supply of the bone.

The author studied the significance of spastic manifestation in the vessels by attempting to prevent as well as to treat the initial stages of osteomyelitis with the aid of repeated administration of hexamethonium and trasentine. Administration of ganglion-blocking substances prevented the development of osteomyelitis in 90% of cases. The osteomyelitic process was controlled in a number of instances. The results demonstrate the importance of the vascular spasm in the pathogenesis of experimental osteomyelitis.

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FIXATION OF SHEEP'S RED CELLS BY THE LYMPHATIC GLANDS OF CATS AND DOGS

N. V. Medunitsyn

From the Department of Pathological Physiology (Head – Corresponding Member AMN SSSR Prof. A. D. Ado) of the N. I. Pirogov Second Moscow State Medical Institute (Director – Docent M. G. Sirotkina)

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From information in the literature, all substances with a molecular weight over 20,000-30,000 are absorbed exclusively through the lymphatic vessels [10]. Antigens which pass through the lymphatic glands become fixed there. The degree of fixation depends on the dispersion of the substances.

There is indirect evidence that foreign proteins are held up with difficulty by lymphatic glands [15, 17]. The smallpox virus passes freely through the lymphatic barrier into the blood stream [16].

There is no unanimity on the fixation of bacteria. Some authors [6, 7] regard the lymphatic system and, hence, the lymphatic glands as conductors of infection. The majority of workers hold the opposite opinion.

Large particles are retained with particular intensity in the lymphatic glands. During perfusion of the popliteal lymphatic glands of the dog with a suspension of isogenous red cells, 99% of them were held up [11].

The mechanism of fixation of large corpuscular antigens is one of mechanical holding up of the antigen by the reticular tissue of the sinuses of the lymphatic glands and of subsequent phagocytosis.

Bearing in mind the role of the lymphatic glands in the process of antibody formation, it may be assumed that they take part in the process of fixation of a specific component – in the reaction of combination of antigen and antibodies within the lymphatic gland itself [8, 9, 12, 13, 14].

The barrier properties of lymphatic glands depend on various conditions: on the reactive state of the body, on the pressure of the lymph entering the gland, on the quantity of antigen reaching it, and so on. In response to immunization there is a significant increase in the barrier function of the lymphatic glands [1,2,3,5].

In the present research we studied the barrier function of the lymphatic glands in relation to the fixation of foreign red cells.

EXPERIMENTAL METHOD

In this investigation we used the method of perfusion of the lymphatic vessels [4] of the popliteal lymphatic glands of dogs and cats with an antigen solution. Estimation of the antigen content of the original fluid and of the fluid after passing through the lymphatic glands enabled the barrier function of the lymphatic glands to be judged with accuracy.

As anesthetic we used a 10% solution of evipan, which was injected into the cats in a dose of 1 ml/kg intramuscularly, and into the dogs in a dose of 0.25 ml/kg intravenously. Furthermore the dogs were given a preliminary injection of a 1% solution of morphine hydrochloride in a dose of 1 ml/kg, 15-20 minutes beforehand.

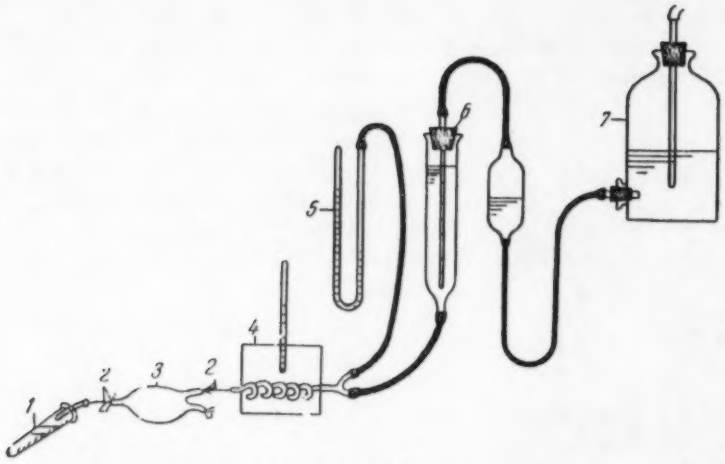

Fig. 1. Diagram of the apparatus for perfusion of a lymphatic gland. 1) Graduated test tube; 2) needles inserted into the lymphatic vessels; 3) lymphatic gland; 4) bath (temperature 38-39°); 5) water manometer; 3) measuring cylinder; 7) Marriott's bottle.

A suspension of sheep's red cells (50,000-60,000/mm^3) in Tyrode solution was used for the perfusion. In order to prevent sedimentation of the red cells, a glass tube, through which air was blown, was introduced into the closed vessel containing the fluid, preventing sedimentation of the red cells and maintaining a constant perfusion pressure (see diagram in Fig. 1).

The pressure of the perfusate was 20, 25 and 30 mm of water. At a lower pressure the percentage fixation of the sheep's red cells was very high. Corresponding to the three levels of pressure, three samples of perfusate were obtained, each of which was collected for 15 minutes. The lymphatic glands on both sides were examined.

After the experiment a solution of Chinese ink was injected through the afferent vessel into the lymphatic gland in order to reveal any possible anastomoses between the afferent and efferent lymphatic vessels.

The number of red cells in the original fluid and in the perfusate was determined by counting in a Goryaev chamber. The barrier function of the lymphatic glands was calculated as the percentage retention of sheep's red cells.

Immunization of the animals was carried out by means of five injections, every 5 days, of a 5% suspension of sheep's red cells in a dose of 1 ml/kg for the cats and 0.3 ml/kg for the dogs. In one group of animals the red cell suspension was injected subcutaneously into the lower part of the calf, and in the other group – into the lateral surface of the body. Investigation of the barrier function of the lymphatic glands was carried out on the 2nd-16th day after completion of immunization.

Altogether 35 cats and 10 dogs were used in the experiments. The number of lymphatic glands perfused was 72 (56 in cats and 16 in dogs).

EXPERIMENTAL RESULTS

The results obtained from perfusion of the lymphatic glands of the cats and dogs were of the same type.

The percentage fixation of sheep's red cells by the lymphatic glands of the unimmunized animals at a perfusion pressure of 20 cm of water varied between limits of 53.8-95.5%, at a pressure of 25 cm between 48.3-94.5%, and at a pressure of 30 cm between 36.0-93.1% (Fig. 2).

The variations in the percentage fixation of sheep's red cells by the lymphatic glands of the immunized cats, injected with the suspension in the region of the calf, were as follows: at a perfusion pressure of 20 cm water 93.1-100%, at a pressure of 25 cm 88.7-100% and at a pressure of 30 cm 78.7-100%.

Thus with an increase in the pressure of the perfusate the percentage fixation of red cells decreased. The degree of lowering of the percentage fixation in response to a change in the pressure of the perfusate was less pronounced in animals immunized in the paw than in intact cats.

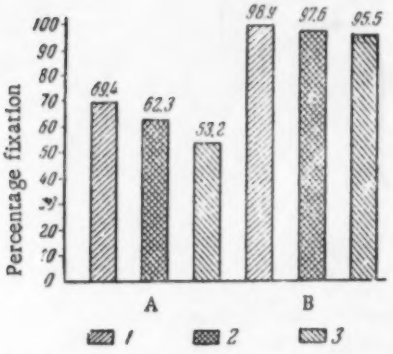

Fig. 2. Fixation of sheep's red cells by the lymphatic glands of the cat at different pressures of perfusate (in percent; mean values given. A) Unimmunized cats; B) immunized (in the region of the calf) cats. 1) At a perfusate pressure of 20 cm of water; 2) at a pressure of 25 cm of water; 3) at a pressure of 30 cm of water.

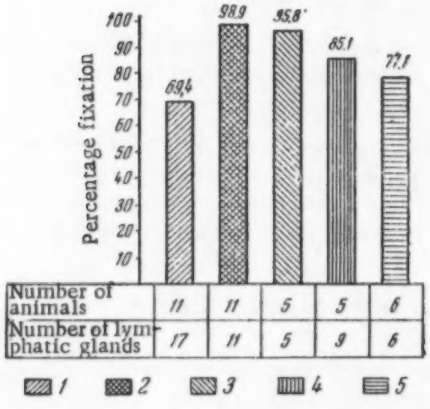

Fig. 3. Fixation of sheep's red cells by the lymphatic glands of the cat (in percent; mean values given. 1) In unimmunized animals; 2) in animals immunized with sheep's red cells (in the region of the calf on the side of immunization); 3) in the same animals, on the opposite side; 4) in animals immunized with sheep's red cells in the lateral region of the body; 5) in animals immunized with dogs' red cells in the region of the calf.

During perfusion of the lymphatic glands at constant pressure, and in some cases even at increased pressure, the percentage fixation gradually rose and the volume of perfusate obtained in each 15 minutes diminished. This phenomenon probably arose as the result of rapidly developing edema and mechanical blocking of the reticular filter of the lymphatic gland.

In Fig. 3 is shown the relationship between the percentage fixation of sheep's red cells and the site of injection of the antigen during immunization of the animals (pressure of the perfusate 20 cm of water).

After immunization of the cats with sheep's red cells in the left paw, the percentage fixation of red cells during perfusion of the lymphatic gland on the right also increased (variation between limits of 89.3-100), and after immunization of the cats in the lateral part of the body the percentage fixation varied from 71.7 to 99.3.

The results of the experiments showed that after immunization of the animals, the fixing power of the lymphatic glands, especially the regional glands, was increased. The question naturally arose of the specificity of this phenomenon.

In order to elucidate this question we immunized cats subcutaneously in the region of the paw with dogs' red cells and perfused the lymphatic glands with a suspension of sheep's red cells. The conditions of immunization and perfusion were the same as in the previous experiments. Under these circumstances the percentage fixation at a perfusate pressure of 20 cm of water was 45.9-99.

On these grounds we may postulate the existence of two components of the function of fixation of the lymphatic glands of immunized animals — a specific and a nonspecific.

After immunization of the animals, especially in the region of the calf, hypertrophy of the lymphatic glands was observed. The weight of the glands was doubled or trebled. The area of contact between the antigen and the reticular tissue of the lymphatic gland was enlarged, thereby stimulating phagocytosis and resulting in considerable antigen fixation. In some experiments, during perfusion of the lymphatic glands of immunized animals, hemolysis appeared in the perfusate, especially in the first samples (when the perfusate was allowed to stand for 24 hours in the cold). In addition hemolysis was observed after the perfusate was allowed to stand in a bath (at a temperature of 37°) and complement was added.

SUMMARY

The method of perfusion of popliteal lymph nodes by suspension of sheep red cells was employed in cats and dogs. With increased perfusate pressure a diminuition of the lymph nodes' fixating function of respective sheep red cells was noted.

In immunized animals this relationship is less pronounced. During the process of immunization in animals, the barrier function of the lymph showed a rise. When compared with the immunization effected in other areas of the body, the fixating function was appreciably higher in the animals inoculated into the region lying below the given lymph node.

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BIOCHEMISTRY AND BIOPHYSICS

THE EXISTENCE OF GLOBULIN X AS AN INDIVIDUAL PROTEIN

I. I. Ivanov, V. V. Kadykov and V. A. Yur'ev

From the Department of Biochemistry of the Leningrad Institute of Pediatric Medicine

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In 1933 Weber [9, 10] suggested the name globulin X for that part of the protein of expressed muscle juice which is precipitated after prolonged dialysis of the juice against distilled water. The same protein, according to Weber's findings, is present also in saline extracts of striped muscles of both high and low ionic strength.

If pulped muscle is extracted with 0.6M KCl at pH = 8.2, and the extract is then dialyzed against a highly diluted phosphate buffer, when the ionic strength of the solution reaches a sufficiently low value, myosin (actomyosin) is the first to be precipitated. With a 0.04M concentration of CKl and at pH = 7.0, myosin (acetomycin) may be completely separated from the other muscle proteins. With a further fall in the concentration of KCl (below 0.005M) globulin X begins to be precipitated. Proteins of the myogen group and myoalbumin remain in solution. Globulin X belongs, therefore, to the group of water-insoluble proteins and, in practice, is a usual component of the muscle protein fraction extracted from pulped muscle by solutions of low ionic strength.

The total content of globulin X obtained by this method, according to Weber's findings, is about 20% of all muscle proteins. In expressed muscle juice, in the nitrogen of the globulin X is contained about 12-13% of the total protein nitrogen. The globulin X precipitated during dialysis may be redissolved only partially, probably because of the partial denaturation of the protein at the time of dialysis.

In spite of the great length of time elapsing since the description of globulin X by Weber, little is yet known about the nature and properties of this protein. According to the findings of Weber and Meyer [9, 10], globulin X possesses normal low viscosity, does not show double diffraction nor form threads when a solution of it is forced through capillary tubes into distilled water. Its isoelectric point is about 5, and its molecular weight, according to Deuticke [5], is 140,000-180,000.

On electrophoregrams globulin X is a component of the myogen fraction. On electrophoresis of muscle extracts with low ionic strength, no special component was found which corresponded in its properties to this protein [3]. Connel [4], by means of electrophoresis, subdivided the globulin X fraction of the muscles of the trout into three components.

In the present paper we describe the results showing the quantitative content of globulin X in muscle juice obtained by expression of the striped muscles of embryos, newborn and adult rabbits, the percentage content of globulin X in the different fractions of muscle juice, salted out by means of ammonium sulfate, and also the results of electrophoretic examination of the water-insoluble muscle globulins.

EXPERIMENTAL METHOD

In order to obtain muscle juice we used the striped muscles of the rabbit (spinal muscles and muscles of the limbs). Separate experiments were performed on the muscles of the heart and stomach. The excised muscles were freed from visible fat and connective tissue septa, washed to remove blood with cold physiological saline and pulped in a mincing machine.

The pulped muscle was immersed in an ice-salt mixture at a temperature of 10-12° until it was frozen to a firm consistency. The frozen pulp was cut into small pieces with a scalpel – "shavings" – ground in a cold mortar into a snow-like mass, and then expressed in a metal mold, also previously cooled.

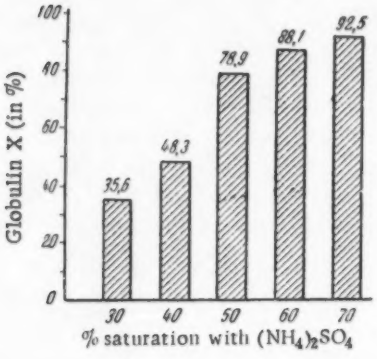

Fig. 1. Amount of globulin X in the precipitate after salting out the proteins of muscle juice with ammonium sulfate (in % of the total globulin X content of muscle juice).

Expression of the muscle juice was conducted at first under low pressure, which was then gradually increased to 50-60 atmospheres. The muscle juice thus obtained was dialyzed in a collodion bag against distilled water for 48 hours at a temperature around 0°. The precipitate – globulin X – was separated by centrifugation and washed three times with cold distilled water. In order to obtain a solution of globulin X the precipitate was treated with a buffer solution with pH = 5.8 and an ionic strength of 0.3.

To shed light on the question at what concentrations of $(NH_4)_2SO_4$ salting out of globulin X took place, saturated ammonium sulfate solution was added to 5 samples of muscle juice (each of 6 ml) in volumes so that the final concentration of $(NH_4)_2SO_4$ in the test samples was 30, 40, 50, 60 and 70% saturation respectively. The salt solution was added to the muscle juice gradually, with slow agitation. Salting out took place in wide test tubes for 12 hours at a temperature from 1° to 4°.

The protein precipitate was separated by filtration through filter paper and was then completely redissolved in a phosphate buffer with pH = 5.8 and ionic strength 0.3.

The globulin X content was determined in the filtrates and precipitates thus obtained, for which purpose a known volume of filtrate and dissolved precipitate was dialyzed for 48 hours against distilled water at a temperature of around 0°. The amount of globulin X precipitated was determined by a gravimetric method (after drying to constant weight at a temperature of +105°) or by the micro-Kjeldahl method after redissolving the protein precipitate.

By means of paper electrophoresis the fractional composition of the proteins of the crude muscle juice, of the muscle juice after removal of the globulin X from it by dialysis, and of the globulin X obtained by dissolving the precipitate formed by dialysis of the muscle juice against distilled water in a buffer solution with pH = 5.8 and an ionic strength of 0.3, was investigated.

Electrophoresis was carried out for 18 hours at a current strength of 0.8 ma per cm cross section of the paper strip and at a voltage of 6-8 v/cm.

EXPERIMENTAL RESULTS

According to our findings, the muscle juice of the striped muscle of the adult rabbit contained 9-10% of protein; the globulin X fraction accounted for about 13% of the total protein content of the muscle juice.

After being precipitated during dialysis, globulin X lost its solubility to a large extent. For instance at pH = 5.8 and an ionic strength of 0.3, only about 40% of the precipitate of globulin X was dissolved, and the remainder lost its solubility under these conditions. This fact agreed with the results of Weber's research.

The globulin X content of muscle juice, according to our findings, depended on the age of the animal and on the type of muscle. The muscle juice of cardiac muscle contained a larger amount of globulin X than the striped muscle (about 20%). In the juice expressed from the muscle of the stomach the globulin X content showed wide variations (from 15 to 30-35%). Characteristic changes in the globulin X content were observed in the muscle juice of the striped muscles in ontogenesis (see table).

In Fig. 1 are shown the results of the experiment in which globulin X was salted out from the muscle juice of the striped muscles of the adult rabbit at different degrees of saturation with ammonium sulfate, from which it may be seen that the smallest quantity of globulin X (35.6% of its total content in the muscle juice) was salted out at 30% saturation with ammonium sulfate. At higher degrees of saturation the amount of globulin X

salted out gradually increased. At 70% saturation 92.5% of the globulin X was precipitated, and even at this degree of saturation it was not fully salted out. The largest amount of protein, globulin in character, was precipitated in the zone of 40-50% saturation with ammonium sulfate (Fig. 1).

Globulin X Content of the Muscle Juice of the Striped Muscle of the Rabbit (as a percentage of the total protein content of the juice examined)

Embryo (25 days)	Newborn	Adult
42	35	13

Globulin X was salted out from the muscle juice at all concentrations of $(NH_4)SO_4$ as a mixture with other proteins. The absolute amount of slated out globulin X rose with an increase in the concentration of ammonium sulfate, whereas the relative content decreased under these circumstances. The highest percentage content of globulin X in the precipitate was at 30% saturation. There was thus no doubt that the globulin X did not have a definite enough zone of salting out.

In Fig. 2 are shown the electrophoregrams of the proteins and the corresponding graphs of crude muscle juice, muscle juice after removal of the globulin X by dialysis and the globulin X preparation.

Several proteins are known to enter into the composition of muscle juice. On the electrophoregram (Fig. 2, a) are seen four main bands, one of which is faintly marked and situated at the starting line, and evidently corresponds to the portion of the most readily denatured protein fraction. Since the ionic strength of the muscle juice did not exceed 0.2, only "water-soluble"* muscle proteins could be present in the expressed juice.

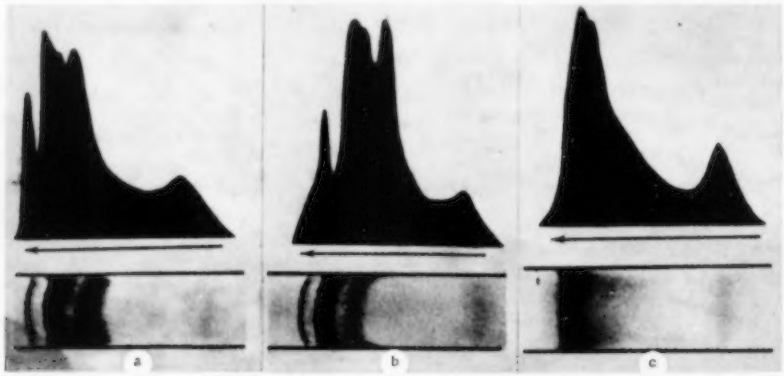

Fig. 2. Electrophoregrams: a) of the proteins of muscle juice of the striped muscles of the adult rabbit; b) of the proteins of muscle juice of the striped muscles of the adult rabbit after removal of the globulin X; c) of the globulin X isolated from the muscle juice of the striped muscles of the adult rabbit.

According to the findings of I. I. Ivanov and his co-workers [1], muscle juice contains neither myosin nor actomyosin. There are also reports [2, 6, 7, 8] that in muscle extracts with an ionic strength of 0.15, during free electrophoresis, three groups of "water-soluble" proteins are revealed, each of which consists of several components (m, n, l, first group; K_1 and K_2, second group; J, Sp, h, third group).

At the present time it is not yet possible to assert definitely to which components of the proteins, soluble in saline media with a low ionic strength, obtained by means of free electrophoresis, correspond all the stains which appear during paper electrophoresis of muscle juice.

* By the term "water-soluble" proteins we designate proteins soluble in saline media with a low ionic strength.

In all probability, judging by the quantitative content and by the order of arrangement on the strip of paper, the three fractions of muscle juice of the adult rabbit (Fig. 2, a) correspond to the components h and m, n and l, and possibly k. Myoalbumin (h) may be found in a significant amount only on the electrophoregrams of the muscle juice proteins of embryonic and newborn rabbits (Fig. 3).

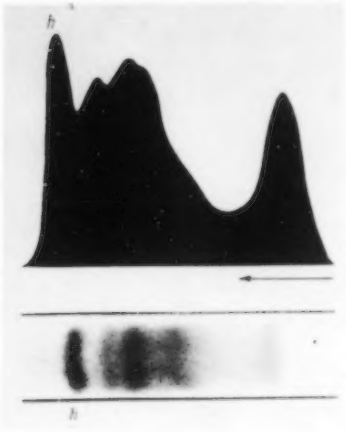

Fig. 3. Electrophoregram of the proteins of the muscle juice of the striped muscles of a newborn rabbit.

After removal of the globulin X from the muscle juice, not one of the fractions disappeared from the electrophoregram (Fig. 2, b). The graphs of the two electrophoregrams are hardly distinguishable from each other; the peak corresponding to the "starting" fraction was somewhat lowered after removal of the globulin X.

Globulin X obtained by redissolving the precipitate from dialysis of muscle juice against distilled water in a buffer solution with pH = 5.8, was found to consist of two main fractions – the larger of them evidently nonhomogeneous, which is shown clearly on the graph (Fig. 2, c). It must also be emphasized that globulin X may possibly have a still more complex fractional composition, since the electrophoretic investigation was carried out only with the soluble part of this protein.

It thus follows from our findings that globulin X does not exist as an individual protein.

The protein described by Weber under the name globulin X is in fact a mixture of globulins with different physiological properties. These proteins do not have a sufficiently definite zone of salting out (in the presence of ammonium sulfate) and their electrophoretic mobilities, although close, are nevertheless not identical.

A certain number of proteins which differ from the true globulins by their solubility in a half-saturated solution of ammonium sulfate enter into the composition of Weber's globulin X.

SUMMARY

The quantitative content of globulin X was investigated in the muscle juice obtained by pressing the striped muscles of embryos, newborn and adult rabbits. The globulin X percentage was also determined in various fractions of the muscle juice salted out with ammonium sulfate. Electrophoretic investigation of water-insoluble muscle globulins was also conducted. The data obtained led the authors to a conclusion that globulin is not an individual protein but a mixture of different globulins varying according to their physicochemical properties. Electrophoretically, globulin is not detected as a separate component. Its content in the striped muscle juice changes during the process of ontogenesis.

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THE UPTAKE OF RADIOACTIVE PHOSPHORUS (P^{32}) BY VARIOUS STRUCTURES OF THE HEART

V. V. Frol'kis and V. I. Mil'ko

From the Department of Physiology (Head – Active Member AN Ukrainian SSR Prof. G. V. Fol'bort) and the Department of Radiology (Head – Docent N. F. Zarkevich) of the Kiev Medical Institute

(Received July 22, 1958. Presented by Active Member AMN SSSR B. N. Man'kovskii)

The whole complexity and diversity of the cardiac activity are determined by a clear interrelationship of its structures, which possess different functional characteristics. The principal functional properties of cardiac muscle – automatism, excitation, conduction, contractility – are inherent to a varying degree in different structures of the heart, and only under pathological conditions do radical changes develop in these characteristics, so that the functional interrelationships of the divisions of the heart are disturbed. The generally accepted view now is that the heart is a functionally nonhomogeneous excitatory system [2, 9, 10, 11, 12, 15, 16].

There is no doubt that the functional characteristics of the structures of the heart are determined by the specific biochemical processes which take place in them. In the study of the biochemical transformations in the heart, however, the specific metabolic processes in its structures have not yet been taken into consideration. By this approach, all the distinctive features of metabolism inherent in the various divisions of the heart, its neuromuscular apparatus, conducting system and nerve ganglia are obliterated.

We consider that the radioactive isotope method, by making it possible to study biochemical conversions even in microstructures, will enable the characteristic features of the metabolism of the different elements of the heart to be compared. In our first move toward the solution of this problem it was important to determine the rate of uptake of labeled atoms in the divisions of the heart – the right and left atria, the right and left ventricles – and in the individual links of the conducting system of the heart – the sino-auricular and atrioventricular nodes and the bundle of His. Bearing in mind the importance of high-energy phosphorus compounds in metabolic processes in the heart, we studied the uptake of radioactive phosphorus in the different structures of the heart.

EXPERIMENTAL METHOD

Experiments were carried out on 28 rabbits. The animals received intravenous injections of 50 μC (16 experiments) or 150 μC (12 experiments) of radioactive phosphorus.

After different intervals of time – 4, 24, 48 and 96 hours – the animals were killed and the heart taken out. Equal samples by weight were taken from the various divisions of the heart. The correct selection of the weighed samples for the investigation was checked morphologically – sections were prepared from corresponding areas of the heart. In order to determine the radioactivity of the areas, in some cases the weighed sample was chopped up into small pieces on the target, in an area of 5 × 5 mm, after which the number of impulses was counted by means of a B-2 counting apparatus. In other cases samples weighing 30-50 mg were incinerated together with 300 mg of starch in a muffle furnace, then evenly distributed on the target, and two drops of celloiden were then allowed to fall on the resulting preparation. No difference in principle was observed between the results in each case.

EXPERIMENTAL RESULTS

The general conclusion from our investigations was that a definite topography existed in the uptake of P^{32} in the various structures of the heart. The most intensive uptake of radiophosphorus took place in the muscle of the left ventricle. This fact was confirmed in all the experiments. Another important discovery was that the radioactivity of the left ventricle greatly exceeded the radioactivity of the blood and of all other divisions of the heart, including the right ventricle. The intensity of uptake of radioactive phosphorus in the left ventricle now and then was 2-3 times greater than in the other divisions of the heart. The comparative data of the intensity of uptake of P^{32} in the various divisions of the heart are shown in Fig. 1.

As may be seen from this figure, in the majority of experiments the following sequence was observed in the intensity of uptake of P^{32} in the division of the heart: left ventricle, right ventricle, left atrium, right atrium. In experiment No. 4, for instance, the number of impulses per minute over and above the background level from a sample taken from the left ventricle was 730, from the right ventricle 400, the left atrium 240 and the right atrium 186; in experiment No. 11 the corresponding figures were: left ventricle 918, right ventricle 598, left atrium 502 and right atrium 400.

In all the experiments we also determined the number of impulses in the elements of the conducting system — the sino-auricular and atrioventricular nodes and the bundle of His. The fact that the sino-auricular and atrioventricular nodes showed low radioactivity was of importance in principle. The intensity of uptake of P^{32} in the nodes of automatism was minimal — significantly less than in all the other structutures of the heart. P^{32} was taken up more intensively by the elements of the bundle of His.

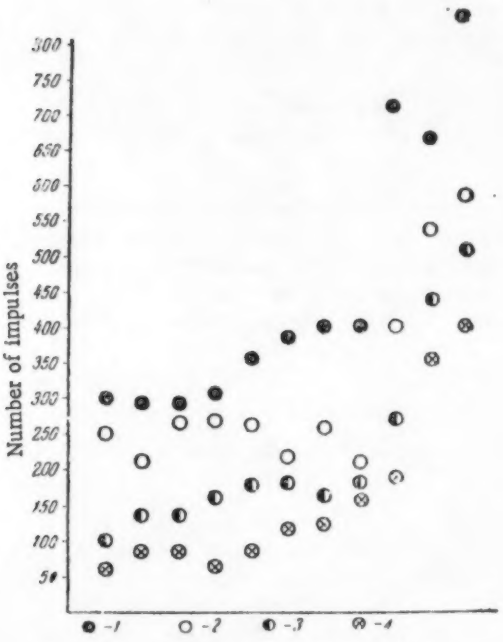

Fig. 1. Intensity of uptake of P^{32} in the left ventricle (1), the right ventricle (2), the left atrium (3) and the right atrium (4).

In Fig. 2 are shown the results of experiments enabling the intensity of uptake of P^{32} by the different structures of the heart to be compared.

It might have been assumed that the topography of distribution of radioactive phosphorus which we found in the heart was largely determined by differences in the blood supply of the heart structures [1, 4, 5, 6, 7, 8, 13] and by the resulting inequality in the blood content of the samples. Both direct and indirect proof was present of the groundlessness of this assumption. In the first place, the radioactivity of some structures of the heart was considerably higher than that of the blood; in the second place there was no parallel between the abundance of the blood supply of the various divisions of the heart and the activity of uptake of P^{32} therein.

In order to prove the relationship between the uptake of P^{32} by the structures of the heart and the intensity of the metabolic processes in these structures, we carried out a series of experiments. The heart of experimental animals was perfused for a long time with physiological saline through a cannula introduced into the aorta. By this means the blood was thoroughly washed from the system of coronary vessels. The intensity of uptake of P^{32} by the various structures of the heart was then determined. The results of these experiments are shown in Fig. 3. We can see that flushing out the coronary vessels did not alter the topography of the intensity of uptake of P^{32} by the structures of the heart.

The known morphological and functional characteristics of the structures of the heart were thus reinforced by our findings of the specificity of the course of the metabolic processes taking place within them. From our point of view a fact of particular interest is that of the minimal uptake of P^{32} by the sino-auricular and atrioventricular nodes, the functional peculiarity of which is their power of automatic activity, which was more pronounced.

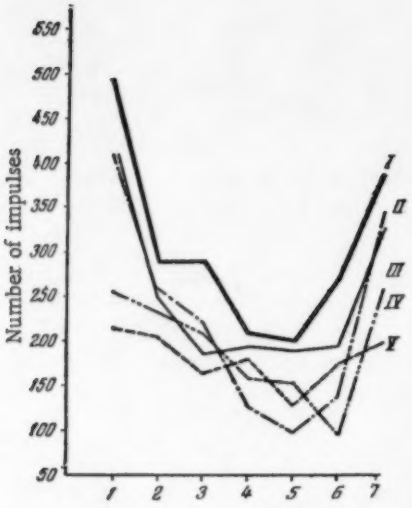

Fig. 2. Intensity of uptake of P^{32} in the different structures of the heart. 1) Left ventricle; 2) right ventricle; 3) left atrium; 4) right atrium; 5) sino-auricular and 6) atrioventricular nodes; 7) bundle of His.

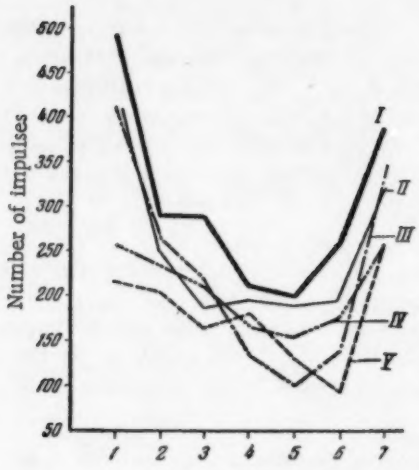

Fig. 3. Intensity of uptake of P^{32} in the different structures of the heart after perfusion of the organ. 1) Left ventricle; 2) right ventricle; 3) left atrium; 4) right atrium; 5) sino-auricular node; 6) atrioventricular node; 7) bundle of His.

in the sino-auricular node. The intensity of uptake of P^{32} by the sino-auricular node was always greater than that of the atrioventricular. The high radioactivity of the bundle of His agrees with the physiological data of its well marked conducting power and contractility.

The information which we gained on the uneven intensity of uptake of P^{32} by the various divisions of the heart – the right and left atria, and the right and left ventricles – indicates the fine adaptation of the metabolic processes to the conditions of normal cardiac activity. Further study of the course of the metabolic processes in the structures of the heart will permit an explanation of the most intimate mechanisms responsible for determining the varied nature of the reaction of the heart to the constantly changing conditions of existence of the animal.

SUMMARY

Experiments were performed on rabbits. There was a definite topography of radiophosphorus (P^{32}) inclusion into different portions and structures of the heart.

The intensity of this inclusion decreased in the following sequence: left ventricle (maximal intensity), right ventricle, left auricle, right auricle. It was also demonstrated that in experimental conditions the radioactivity of the left ventricle is much greater (by 3-4 times) than that of the other portions of the heart and blood. The centers or nerve ganglions governing automatic control are characterized by a minimal intensity of the P^{32} inclusion. The inclusion of radiophosphorus is more intense in the elements of the bundle of His than in the sino-auricular and atrioventricular nodes.

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CHANGES IN THE ASCORBIC ACID CONTENT OF THE INTERNAL ORGANS OF WHITE RATS IN RESPONSE TO THE ACTION OF CHLORINATED HYDROCARBONS

COMMUNICATION II. THE EFFECT OF METHYLENE CHLORIDE

I. P. Ulanova and B. I. Yanovskaya

From the Group of Active Member AMN SSSR B. A. Lavrov and the Industrial Research Laboratory (Head – Prof. Z. B. Smelyanskii) of the Institute of Work Hygiene and Occupational Diseases (Director – Active Member AMN SSSR A. A. Letavet) of the AMN SSSR, Moscow

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It was shown in the previous communication [3] that the acute and repeated action of dichloroethane vapor causes severe changes in the rate of biosynthesis of ascorbic acid in rats and that under these circumstances characteristic changes develop in the ascorbic acid concentration in the animal's organs.

Information in the literature on the toxicology of methylene chloride is comparatively scanty and indicates its marked narcotic action [2, 4, 12] and degenerative changes which it produces in the parenchymatous organs.

Gross [8], for instance, after repeated poisoning of rats by exposures of 8 hours to the vapor of methylene chloride in concentration of 4-6 mg/l, found morphological changes only after 75 days, consisting of slight atrophy and central lobar fatty degeneration of the liver. In other research [10], by the action of methylene chloride in a concentration of 34 mg/l for 4 hours per week over a period of 7½ weeks only a weak or moderate degree of fatty degeneration of the liver was observed in a proportion of the experimental animals.

Morphological investigations carried out on the organs of mice exposed to a single inhalation of methylene chloride in a concentration of 45 mg/l, and dying during the exposure, showed well-marked congestion of all the internal organs. In the liver swelling of the reticulo-endothelial cells was observed, and in some cases proliferation of these cells and their infiltration with tiny droplets of fat. In the kidneys slight signs of parenchymatous degeneration of the epithelium of the convoluted tubules were seen, and in the heart, proliferation of histiocytic cells and interstitial tissue. In the brain there was, besides congestion, capillary stasis, signs of perivascular and pericellular edema, acute swelling of the protoplasm of the nerve cells of the cortex, the subcortical ganglia, the brain stem and the other divisions of the brain. In animals dying a few days after poisoning, the changes in the organs were more pronounced than in the animals which died during exposure.

There are isolated reports of toxicoses in man from the action of methylene chloride.

Klotzbücher [9] reported poisoning of 10 persons, one of whom died. These patients developed loss of consciousness with, in some cases, tonic and clonic convulsions, absence of pupillary reactions and quickening of the pulse and respiration. Moskowitz and Shapiro [11] described the acute poisoning of 4 workmen from inhalation of methylene chloride vapor under industrial conditions.

During examination of workmen using methylene chloride, disturbances were found both in the nervous system and in the internal organs. Their main complaints were dizziness, headaches, pains in the region of the

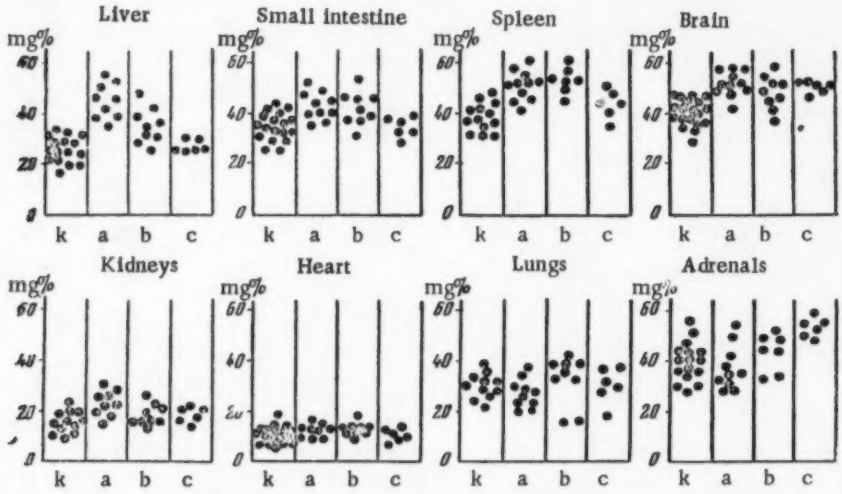
heart and a pricking sensation in the eyes. From their medical histories, in acute methylene chloride poisoning, patients had a sensation of intoxication, with severe dizziness, vomiting and general weakness.

In the present communication we give data on the action of this member of the group of chlorinated hydrocarbons – methylene chloride – used in several industrial processes and, in particular, in the manufacture of cinefilm.

We investigated the changes in the ascorbic acid concentration in the organs of rats weighing 180-200 g subjected to acute and repeated exposure to methylene chloride.

EXPERIMENTAL METHOD

Two series of experiments were carried out to study methylene chloride poisoning under static conditions. In the first series of experiments 20 rats were subjected to acute poisoning with methylene chloride in a concentration of 40 mg/l by exposure for 2 hours. The ascorbic acid content of the organs of animals, killed by decapitation, was investigated one and 24 hours after poisoning. In the second series of experiments we studied



Ascorbic acid content in the organs of rats after inhalation of methylene chloride. k) Control; a) methylene chloride, 40 mg/l, one exposure, killed after 1 hour; b) methylene chloride, 40 mg/l, one exposure, killed after 24 hours; c) methylene chloride, 0.7 mg/l, repeated exposure, killed after one month.

the action of low concentrations of methylene chloride (0.6-0.7 mg/l) after repeated poisoning for 2 hours a day, 6 times a week, for one month. Experimental animals were killed one hour after the end of the last exposure.

As controls we used 20 rats of the same sex and weight, kept under identical conditions, but not exposed to the action of methylene chloride. The ascorbic acid concentration in the liver, small intestine, spleen, cerebral hemispheres, kidneys, adrenals, heart and lungs of these animals was determined immediately after death.

The control animals were examined at the same times as the experimental. The results obtained were subjected to statistical treatment.

EXPERIMENTAL RESULTS

In response to the action of methylene chloride vapor in a concentration of 40 mg/l, the animals developed a clear picture of poisoning, with disturbances of the coordination of movements, dragging of the limbs, lying on the side and, finally, narcosis. After the conclusion of the exposure to the poison the animals quickly recovered, and 40-50 minutes later they appeared outwardly healthy.

In the second series of experiments, in response to repeated poisoning the behavior of the animals in the poison chamber was normal, and no clinical signs of methylene chloride poisoning were present.

The results obtained showing the ascorbic acid concentration in the organs are given in the figure.

In the animals poisoned by one exposure to methylene chloride in a concentration of 40 mg/l, after one hour a sharp increase in the ascorbic acid concentration was observed in the very great majority of organs examined, with a very high value for the significance of the difference from the control figures. In the liver, for instance, the ascorbic acid concentration increased by 70% (from 26.1 to 44.4 mg%), in the small intestine by 25% (from 34.9 to 43.8 mg%), in the spleen by 40% (from 39.3 to 57.7 mg%), in the cerebral hemispheres by 29% (from 40.5 to 52.1 mg%), in the heart by 30% (from 9.5 to 12.4 mg%) and in the kidneys by 20% (from 17.4 to 21.1 mg%).

The increase in the ascorbic acid concentration in the majority of organs indicated a general increase in the biosynthesis of ascorbic acid in response to the action of this compound.

Just as in guinea pigs, which do not synthesize ascorbic acid, in analogous conditions the converse occurred — a decrease in the ascorbic acid content of the organs [6, 7]; the increase in the biosynthesis of ascorbic acid in animals which do synthesize this vitamin was evidence of the increased expenditure of ascorbic acid in the poisoned animals, in compensation for which a controlled increase in the rate of biosynthesis of ascorbic acid took place.

The logical deduction from this fact is that, in persons exposed to the action of methylene chloride vapor in industrial conditions, there is increased expenditure of ascorbic acid by the tissues and it is essential to increase the supply of this vitamin.

The increase in the concentration of ascorbic acid in the barrier organs — the liver and kidneys — was evidence of the toxic action of this factor [1, 5]. Its influence on the blood system was reflected by an increase in the ascorbic acid concentration in the spleen. Its severe effect on the central nervous system was shown by the significant increase in the ascorbic acid concentration in the cerebral hemispheres, etc.

The increased concentration of ascorbic acid in all the organs enumerated was still present 24 hours after the single exposure to the poison. In the liver, for instance, by comparison with the controls there was an increase of 26%, i.e., 44% less than one hour after poisoning, and this difference was significant ($T = 4.1$). In the brain and kidneys the increase in the ascorbic acid concentration after 24 hours was also less than in the previous group; in the remaining organs — the intestine, spleen and heart — the ascorbic acid concentration remained at a high level; the action of methylene chloride was also shown by an increase in the ascorbic acid concentration in the lungs.

The changes observed after repeated inhalations of small doses (0.6-0.7 mg/l) of methylene chloride differed from those taking place after a single exposure to a large dose. As may be seen from the figure, this type of poisoning had an effect on the central nervous system (brain) and the adrenal-sympathetic system (adrenals). The increase in the concentration of ascorbic acid in the cardiac muscle was close to significant ($T = 2.5$).

With small doses no marked spread of the toxic action was observed, since no changes were found in the ascorbic acid content of the liver, kidneys, spleen or intestine.

SUMMARY

Acute single action (inhalation) of a high concentration (40 mg/l) of methylene chloride is associated with the rise of ascorbic acid biosynthesis with a sharp increase of its concentration in a number of organs (liver, small intestine, spleen, brain, kidneys, heart, lungs). This reflects the involvement of the corresponding systems of the body into the protective reaction against the toxic effect of methylene chloride. In 24 hours after the inhalation this action is still pronounced, although slightly weakened.

Repeated daily inhalations of methylene chloride (concentration — 0.6-0.7 mg/l) for the period of one month causes a pronounced effect on the central nervous and adrenal sympathetic systems, judging by the dynamics of ascorbic acid content in the organs.

The data obtained demonstrate the increased utilization of ascorbic acid during the action of methylene chloride on the organism and give grounds for concluding that persons subjected to the effect of methylene chloride vapors require supplementary vitamin C administration.

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PHARMACOLOGY

THE EFFECT OF RESERPINE ON THE VEGETATIVE REFLEXES

E. S. Milovidova

From the Laboratory of Special Pharmacology (Head – Active Member AMN SSSR V. V. Zakusov) of the Institute of Pharmacology and Chemotherapy (Director – Active Member AMN SSSR V. V. Zakusov) of the AMN SSSR, Moscow

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Numerous investigations have shown that reflexes from the interoceptors are of fundamental importance in the regulation of the activity of the cardiovascular system. It is also well known that reflex influences from certain receptor fields may take part in the development of different pathological processes, including hypertensive diseases.

In studying drugs used in the treatment of hypertension it is, therefore, important to take into consideration their influence on the interoceptive reflexes.

Hypotensive drugs which have recently become widely used are various preparations obtained from the root of rauwolfia (reserpine, rescinnamine, alseroxylon, rauwiloid and also total rauwolfia alkaloids). Reserpine is particularly widely used in the treatment of hypertension and of several other pathological conditions.

Investigations carried out previously on the effect of total alkaloids of Rauwolfia serpentina on the vegetative serpentina on the vegetative reflexes [2] showed that this preparation depresses the reflexes from the mechano- and chemoreceptors in various receptor fields (the mechanoreceptors of the carotid sinus, the large intestine and the urinary bladder, the chemoreceptors of the pericardium) and also from the central ends of the divided vagus and tibial nerves.

The total alkaloid preparations of rauwolfia is known to contain alkaloids with different, and sometimes directly opposite, types of action. In this connection it was interesting to determine whether the changes which we observed in the interoceptive reflexes were associated with the effect of one of the principal alkaloids contained in rauwolfia – reserpine.

The majority of information in the literature concerns the effect of reserpine on the reflexes from the mechanoreceptors of the carotid sinus and from the central ends of the vagus nerve trunk, divided in the neck, and the sciatic nerve [4, 7, 8].

This research is not of a systematic character and does not permit a full enough idea to be gained of the influence of reserpine on vegetative reflexes, the reflex pathways of which are connected at different levels of the central nervous system. In addition there is an almost complete absence of information on the effect of reserpine on reflexes arising in response to stimulation of chemoreceptors.

The aim of the present work was to study the influence of reserpine on the reflex reactions of the circulation of the blood and respiration arising from the mechano- and chemoreceptors of different receptor fields, namely: the mechanoreceptors of the carotid sinus, the large intestine and the urinary bladder; the central ends of the divided vagus, brachial and tibial nerves, and also from the chemoreceptors of the pericardium and the small intestine.

EXPERIMENTAL METHOD

Experiments were carried out on anesthetized and decerebrate cats. Reflexes affecting the arterial blood pressure and respiration from the mechanoreceptors of the urinary bladder and large intestine were elicited by distension of the organs, reflexes from the mechanoreceptors of the carotid sinus by clamping the common carotid artery. In order to stimulate the vagus, brachial and tibial nerves, rectangular impulses were used from an electronic stimulator (frequency 30-50 cps, duration of impulse 2-2.5 millisec, voltage 0.5-6 v).

In order to elicit reflexes from the chemoreceptors of the pericardium and small intestine we used the method of perfusion of these organs, with their nerve connections with the body maintained. To stimulate the chemoreceptors we used acetylcholine in a concentration of $1:10^{-5}$, $1:10^{-4}$ and in a volume of 0.3-0.5 ml.

The reflexes obtained from the chemoreceptors of the small intestine were pressor in character; the reflexes from the chemoreceptors of the pericardium were either pressor or depressor in different experiments.

The arterial pressure was recorded in the carotid artery by means of a mercury manometer and the respiration by a Marey's capsule, via a tracheal cannula.

In the experiments we used pure reserpine obtained from the All-Union Research Chemopharmaceutical Institute, and also reserpine marketed in tablet form by the firm "SANABO". In view of the very low solubility of reserpine in water, we used the latter in the form of a 0.1% solution in glacial acetic acid.

In order to exclude the possibility of any influence of acetic acid on the reflexes, control experiments were carried out, which showed that acetic acid, when administered in the concentration used for dissolving the preparation, had no essential effect either on the level of the arterial pressure or on the reflexes.

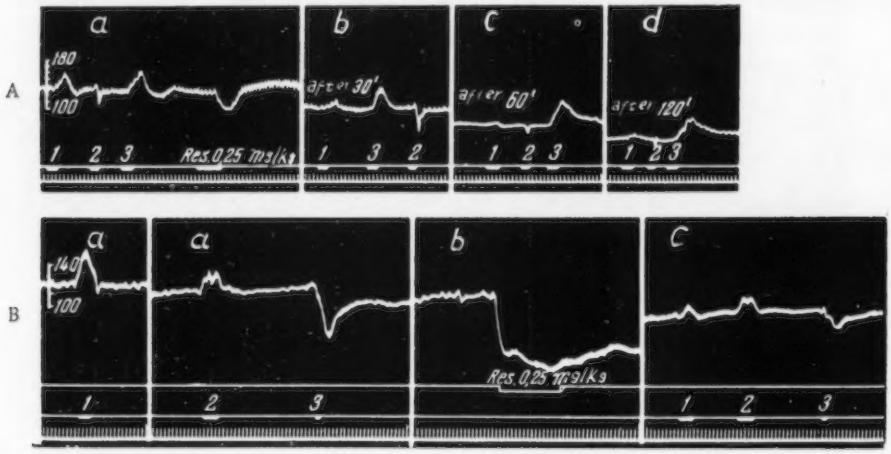

Fig. 1. The effect of reserpine on reflexes from the mechanoreceptors of the carotid sinus, the chemoreceptors of the pericardium and the central ends of the divided vagus and tibial nerves. Legend (from above down): arterial pressure; marker of injection of the drug and stimulation marker; time marker (5 seconds). A: 1) Reflex from the carotid sinus; 2) reflex from the vagus nerve; 3) reflex from the tibial nerve. a) Before injection, and injection of 0.25 mg/kg of reserpine; b) 30 minutes after injection of reserpine; c) 60 minutes after; d) 120 minutes after. B: 1) Reflex from the carotid sinus; 2) reflex from the tibial nerve; 3) reflex from the chemoreceptors of the pericardium. a) Before injection; b) in response to injection of 0.25 mg/kg; c) 60 minutes after injection of reserpine.

In all the experiments the reserpine was injected intravenously.

When the experimental results were being worked out, the change in the magnitude of the reflexes was calculated as a percentage in relation to the initial level of the arterial pressure.

EXPERIMENTAL RESULTS

As the experiments showed, reserpine, when injected in a dose of 0.25 mg/kg, caused a fall in the arterial pressure of 50-60 mm Hg. After 5-10 minutes the pressure was restored to its initial value.

It must be pointed out that the action of reserpine developed gradually, and the arterial pressure, when restored to its initial level, fell again after 30-60 minutes to 30-40 mm below the initial level. The maximum fall in the arterial pressure was observed at the end of the 2nd-3rd hour, and reached 50-70 mm. The fall in the arterial pressure in response to injection of reserpine was also accompanied by marked bradycardia.

From 30 to 60 minutes after injection of reserpine in a dose of 0.25 mg/kg, a clear depression of the reflexes was observed. It must be pointed out that the reflexes from the mechanoreceptors of the carotid sinus were diminished by 50-60%, and those from the mechanoreceptors of the urinary bladder and large intestine by 30-40%.

The reflex on the arterial pressure from the central end of the divided vagus and branchial nerves, like the reflex from the carotid sinus, showed little resistance to the action of reserpine. In a dose of 0.25 mg/kg, reserpine reduced these reflexes by 40-50%. The reflex from the central end of the divided tibial nerve in some experiments not only was not depressed, but was obviously increased (Fig. 1, A).

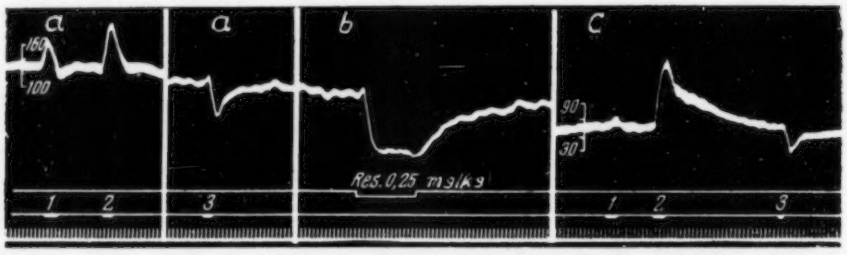

Fig. 2. The effect of reserpine on reflexes from the mechanoreceptors of the carotid sinus, the chemoreceptors of the pericardium and the central end of the divided vagus nerve. Ditilin (a curare-like drug). Legend (from above down): arterial pressure; marker of injection of drug; stimulation marker; time marker (5 seconds). 1) Reflex from the carotid sinus; 2) reflex from the tibial nerve; 3) reflex from the chemoreceptors of the pericardium. a) Before injection; b) injection of 0.25 mg/kg; c) 60 minutes after injection of reserpine.

The reflexes from the mechanoreceptors thus did not possess the same resistance to the action of reserpine. The lowest resistance to reserpine was shown by the reflexes from the central end of the divided vagus nerve and from the mechanoreceptors of the carotid sinus.

When injected in a dose of 0.25 mg/kg, reserpine caused considerable depression of the reflexes on the arterial pressure from the chemoreceptors of the pericardium were diminished by 35-40% and those from the chemoreceptors of the small intestine by 50-55% by comparison with the initial level (see Fig. 1, B).

In a dose of 0.5 mg/kg, reserpine caused a fall in the arterial pressure which lasted more than two hours. At the same time, when reserpine was given in this dose a more marked depression of the reflexes took place, as far as their complete disappearance.

It must be pointed out that the reflexes on respiration arising in response to stimulation of the receptor fields which we studied were more resistant to the action of reserpine than were the reflexes on the arterial pressure. For instance, the reflex on respiration in response to stimulation of the vagus nerve was not at all

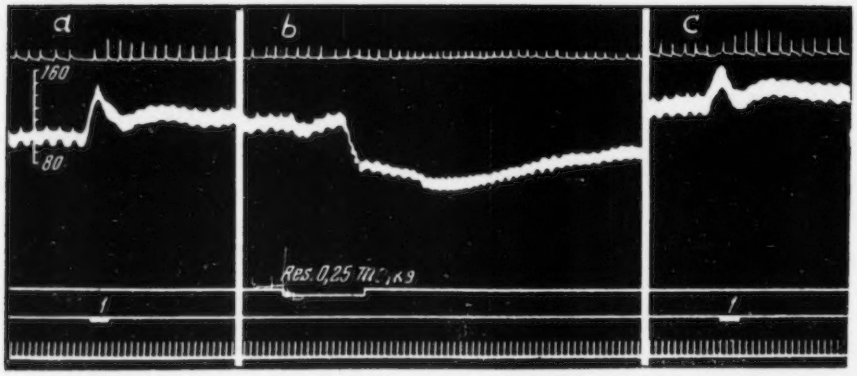

Fig. 3. The effect of reserpine on the reaction of the arterial pressure to stimulation of the peripheral end of the divided splanchnic nerve. Legend (from above down): respiration; arterial pressure; marker of injection of the drug; stimulation marker. a) Background; b) reaction of the arterial pressure and respiration to the injection of 0.25 mg/kg of the drug; c) 60 minutes after the injection.

depressed under the influence of reserpine even when the drug was given in doses which caused complete depression of the reflexes on the arterial pressure.

In spite of the reports in the literature that anesthetics (urethane, nembutal) have a weak action on reflexes from the internal organs [1], we had to exclude the possibility that the effect of reserpine was enhanced by the anesthetics, for reserpine is known to potentiate the action of the latter [9].

For this purpose a special series of experiments was carried out on cats rendered immobile by means of the curare-like drug ditilin. Under these circumstances it was shown that reserpine, in a dose of 0.25 mg/kg, given to cats rendered immobile by ditilin, caused depression of the reflexes from the mechano- and chemoreceptors to a lesser degree than in animals under anesthesia (Fig. 2). Evidently the effect of the action of reserpine on the reflex reactions of the circulation of the blood during anesthesia was somewhat enhanced. However we obtained no essential difference in the action of reserpine in experiments under these particular conditions.

In a special series of experiments we investigated the mechanism of the action of reserpine on the interoceptive reflexes. The generally accepted view nowadays is that the reflexes from the carotid sinus and other receptor fields are effected mainly by the vasoconstrictor fibers of the thoracolumbar division of the sympathetic nervous system and have a common efferent part of the reflex arcs.

The experiments which we carried out showed that the reaction of the arterial pressure in response to stimulation of theperipheral end of the divided preganglionic trunk of the splanchnic nerve after removal of the adrenal gland was preserved when reserpine was injected in a dose of 0.5 mg/kg, i.e., in a dose at which the reflexes were almost completely depressed (Fig. 3).

The control experiments thus led to the conclusion that depression of the interoceptive reflexes by reserpine evidently does not depend on the influence of the drug on the efferent pathways of the vascular reflexes.

The effect of reserpine on the receptors was also studied. For this purpose reserpine, in a dose of 0.25 mg per kg, was injected into the nutrient fluid during perfusion of an isolated loop of small intestine with the nerve connections intact. The experiments showed that the reflex on the arterial pressure from the chemoreceptors of the small intestine was not altered by bringing the reserpine solution into direct contact with the receptors.

The effect of reserpine on the reflexes was thus, evidently, not connected with either its action on the receptor formations or with its action on the efferent pathways of the vascular reflexes.

The results of these investigations thus lead to the conclusion that the effect of reserpine on the interoceptive reflexes is central in character. This hypothesis was confirmed by two other facts.

In the first place, in decerebrate animals the influence of reserpine on these reflexes was much more weakly demonstrated than in the experiments on anesthetized animals. Even when large doses of reserpine (0.5 mg/kg) were given, the depression of the reflexes was much less than was observed in the anesthetized animals.

Further confirmation of the central action of reserpine was given by the selective nature of its influence on the reflexes arising in response to stimulation of different receptor fields.

The reflexes least resistant to the action of reserpine were those from the carotid sinus and the central end of the divided vagus nerve. Furthermore, the reflex from the tibial nerve in some experiments not only was not depressed but was clearly enhanced. This selective action may possibly be due to the fact that reserpine depresses to a lesser degree those reflexes whose afferent centripetal pathways pass through the spinal cord.

It may also be established from these experiments that depression of the vegetative reflexes does not depend on the effect of reserpine on the efferent paths of the interoceptive reflexes and the receptors themselves. It may be postulated that its action on the vegetative reflexes is due to its influence on the central circulatory control system. This point of view is confirmed by reports in the literature, according to which reserpine experts an influence on the hypothalamic region, which is to depress the vascular reactions in response to direct stimulation of the hypothalamus [3, 5, 6].

SUMMARY

Experimental investigations ascertained the selective nature of reserpine action on the interoceptive reflexes. The reflexes from the carotid sinus and the central section of the vagus were the least resistant to the effect of this preparation. In a series of experiments the reflex from the tibial nerve not only shows no inhibition, but becomes markedly intensified.

The experiments also proved that the negetative reflexes are inhibited irrespective of the reserpine effect on the efferent pathways of the interoceptive reflexes and receptor formations. Its effect on the vegetative reflexes is evidently caused by the action upon the central apparatuses of the blood circulation.

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*Original Russian pagination. See C.B. Translation.

THE ACTION OF NARCOTICS ON THE MONOSYNAPTIC REFLEXES OF THE CERVICAL AND LUMBAR REGIONS OF THE SPINAL CORD

O. B. Il'inskii

From the Laboratory of Pathophysiology (Head — the late Prof. V. S. Galkin) and the Laboratory of the Physiology of the Circulation of the Blood and Respiration (Head — Prof. G. P. Konradi) of the I. P. Pavlov Institute of Physiology (Director — the late Academician K. M. Bykov) of the AN SSSR, Leningrad

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Research by a number of workers has shown that the polysynaptic reflex arcs of the lumbar division of the spinal cord are more sensitive to the action of narcotics than the corresponding reflex arcs of the cervical division [2, 3, 6, 7, 13]. The monosynaptic reflexes have received only little study in this direction. On this subject, some authors [13] consider that narcotics depress different monosynaptic reflexes to different degrees, and others [5] point out that bineuronal reflex arcs in different regions of the spinal cord are successively excluded.

The present work was carried out in order to elucidate the action of narcotics on the monosynaptic reflexes of different regions of the spinal cord.

EXPERIMENTAL METHOD

Experiments were carried out on spinal cats. Under deep ether anesthesia the spinal cord was divided at the level of the 1st cervical vertebra, after which the animal was transferred to artificial respiration and was kept warm, and the anesthesia was terminated. Recordings were made of the ipsilateral and contralateral reflexes of the extensors of the elbow (triceps) and knee, which have similar functions and can be classed as monosynaptic reflexes [8-12, 15]. For this purpose steel drills were passed through the humerus and femur, and firmly fixed to a stand. By means of electromagnetic coils with iron cores, rhythmic and synchronous blows of equal strength were applied through the skin to the tendons of the triceps brachii and quadriceps femoris muscles. At the moment of closure of the circuit (once in 3 seconds) the cores were drawn into the coils, thereby striking the tendons, and when the circuit was broken the cores were brought back to their original position by the pull of a rubber band. The isotonic contractions of the muscles were recorded on a kymograph. The initial amplitude of the knee jerk in all the experiments was 2-3 times greater than that of the triceps jerk, so that its recording was reduced in size by the appropriate degree by means of pneumatic transmission.

In some experiments the spinal cord was divided in addition at the level of T9-10 and all the limb muscles were denervated (except the triceps brachii and quadriceps femoris muscles), and the blood pressure in the common carotid artery was recorded.

We investigated the action of sodium amytal, evipan, penthothal, ether, chloralose, ethyl alcohol, chloral hydrate and urethane. Ether was administered by inhalation, using an artificial respiration apparatus, and all the other drugs were injected into the femoral (sometimes the jugular) vein, in solutions preparared ex tempore. The anesthetic drug was administered several times in each experiment. The last injections showed no essential difference from the first in their action.

The results of the more detailed study of the action of pentothal and urethane were treated statistically. They are given in the table, in which each figure is the mean value of several observations, and the limits of

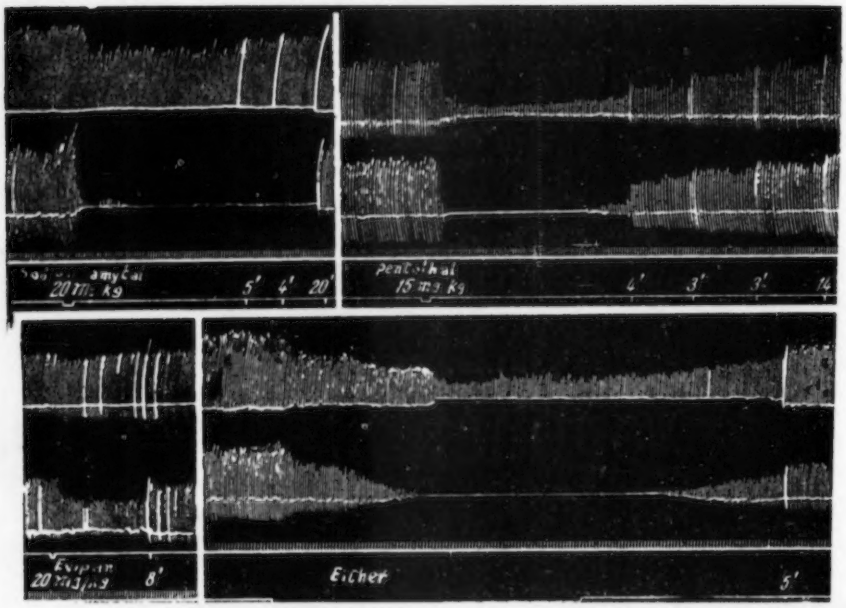

Fig. 1. The action of sodium amytal, evipan, pentothal and ether on the tendon reflexes. Significance of the curves (from above down): extensor reflex of the elbow, knee jerk, time marker (5 seconds), stimulation marker. It is clearly seen that sodium amytal, evipan, pentothal and ether cause, mainly, depression of the knee jerk.

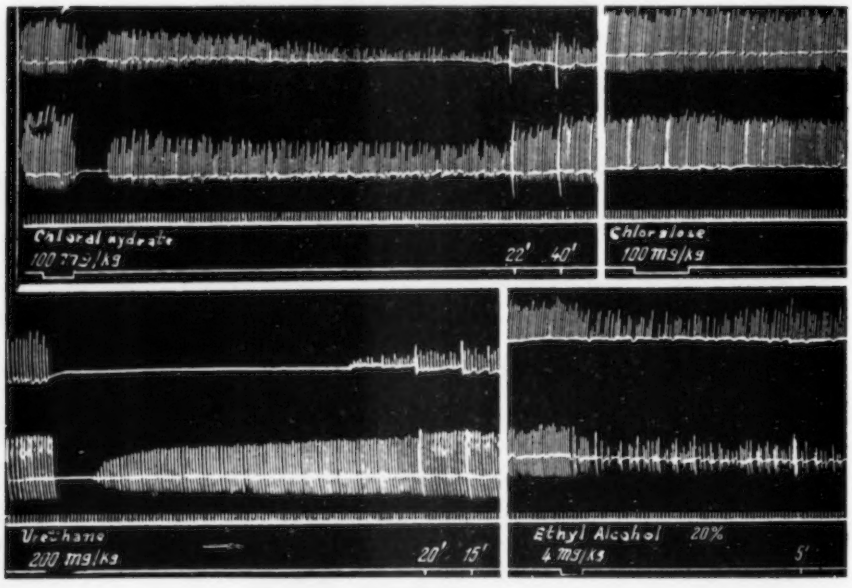

Fig. 2. The action of chloral hydrate, chloralose, urethane and ethyl alcohol on the tendon reflexes. Significance of the curves as in Fig. 1. It will be seen that chloral hydrate and urethane depress, mainly, the extensor reflex of the elbow. Chloralose produces no essential changes, but ethyl alcohol gives a characteristic picture of equal inhibition of the two reflexes under examination.

Changes in the Tendon Reflexes by the Action of Anesthetics

Dose, in mg/kg body weight	Degree of inhibition of reflexes, as % of original amplitude															
	triceps jerk	knee jerk	triceps jerk	knee jerk	triceps jerk	knee jerk	triceps jerk	knee jerk	triceps jerk	knee jerk	triceps jerk	knee jerk	triceps jerk	knee jerk	triceps jerk	knee jerk
	time in minutes (from moment of injection)															
	1/2	1,2	1	1	2	2	3	3	5	5	10	10	15	15	20	20
Pentothal																
20	76. 7	82.1	82.6	83.1	84,5	89.6	83.5	87.6	81.5	77.9	56.8	59.1	46.3	43.3	39.4	39.9
15	44.8	75.7	56.9	81.6	56.6	83.7	53.6	79.4	45.9	68.6	32.5	52.9	21.3	37.2	15,5	26,9
10	12.8	46.7	33.0	61.6	34.7	61.0	32.9	54.5	26.1	53.9	16.4	38.3	12.3	37.9	10.2	27.9
5	0	19.0	5.0	28.0	8.8	28.8	11.3	28.2	7.3	24.6	3.7	16.0	2.6	14.4	2.2	14.1
Urethane																
500	83.2	62.7	54.1	65.8	83.6	83.1	90.3	67 7	84.0	55.2	73.4	37.0	53.6	20.6	40 6	15.1
400	91.8	73.0	95.6	72.3	85.7	64.3	75.9	49.4	57.8	34.8	39.5	20.1	27.5	14.5	20.4	12.6
300	73.1	56.6	74.5	53.6	78.6	46.1	62.1	33.8	45.9	23.8	33.2	16.1	26.6	13.2	24.4	13.4
200	84.5	60.1	82.5	53.7	73.3	38.6	61.1	30.3	52.3	22.6	34.6	16.3	27.5	10.7	19.1	4.3
100	38.2	27.6	38.1	24,2	36.5	21.4	30.5	16.5	25.9	11.9	18.2	9.1	14.9	5.4	11.6	3,2

statistically significant variations in the degree of change of the reflexes are indicated by the bold line. The difference in the degree of inhibition of the reflexes was regarded as significant when $P < 0.05$ [1]. Altogether 246 observations were made on 82 animals.

EXPERIMENTAL RESULTS

The action of the various barbituric acid derivatives – sodium amytal (10-40 mg/kg), evipan (20-40 mg/kg), pentothal (5-20 mg/kg) – on the tendon reflexes investigated was the same. All these drugs produced, mainly, depression of the knee jerk (Fig. 1). The maximum inhibition of the reflexes was observed 2 minutes after injection (see table). In some cases immediately after injection of the barbiturate the reflexes increased slightly (especially the knee jerk), and then became depressed. Recovery of the triceps jerk took place more rapidly than that of the knee jerk.

The action of ether was identical with that of the barbiturates (see Fig. 1), i.e., it selectively depressed the knee jerk. Chloral hydrate and urethane had the opposite action to ether and the barbiturates. Both chloral hydrate (50-100 mg/kg) and urethane (100-500 mg/kg) mainly inhibited the extensor reflex of the elbow (Fig. 2, see table). Under these circumstances certain peculiarities were observed in the action of chloral hydrate. At the moment of injection both reflexes were depressed for a short time; later they recovered rapidly, and only after this did a slowly developing depression appear, which was greater in the triceps jerk. Restoration of both reflexes (especially the triceps jerk) took place much more slowly than in the case of action of the other anesthetics.

Even in quite large doses (50-150 mg/kg), chloralose had practically no action on either reflex (see Fig. 2) or else it gradually depressed both reflexes to an insignificant degree. Ethyl alcohol also altered the essential character of the contractions (see Fig. 2), or depressed the knee jerk to a greater extent. Often when alcohol was given, a considerable increase was observed in the reflexes at the moment of injection.

Experiments were carried out on spinal animals, and therefore the difference in the degree of inhibition of the reflexes examined did not depend on the direct effects of higher divisions of the central nervous system. The character of the reaction was unchanged by the use of the ipsilateral or contralateral limbs, by division of the spinal cord at a level below the thoracic vertebrae and division of all the nerves of the limb apart from those supplying the triceps brachii and quadriceps femoris muscles. No causal connection could be established between the changes in the tendon reflexes and the blood pressure changes and the site of injection of the drug (femoral or jugular vein).

In view of the fact that, other things being equal, the pentothal group and the urethane group had opposite actions, the degree of vascularization of the cervical and lumbar regions of the spinal cord played no essential part in this phenomenon.

It may thus be considered that the differing depth of inhibition of the reflexes which we examined, by anesthetics, depends on certain features of the structure of these reflexes which have not yet been studied.

The subdivision of the anesthetics into groups, which occurred in our experiments, was mainly in agreement with the existing classifications in the literature [4, 14]. An analysis of this subdivision of the anesthetics into groups forms the subject of our later investigations.

SUMMARY

Experiments were performed on spinal cats. The author studied the effect of sodium amytal, evipan, pentothal, ether, chloralose, ethyl alcohol, chloral hydrate and of urethane on the monosynaptic (tendon-elbow extensor and knee) reflexes of the cervical and lumbar sections of the spinal cord.

Sodium amytal, evipan, pentothal and ether inhibit mainly the knee jerk while chloral hydrate and urethane, the elbow extensor reflex. In the doses employed chloralose did not materially change any of the reflexes, while ethyl alcohol either inhibited both reflexes to the same degree, or had a greater inhibitory effect on the knee jerk. Changes in the tendon reflexes under the effect of narcotics were not directly influenced by the higher divisions of the central nervous system, interaction of different areas of the spinal cord, reciprocal relationships, blood pressure variations, the site of administration of the above preparations or by the different degree of vascularization of the cervical and lumbar sections of the spinal cord, but depend on the, as yet uninvestigated, peculiarities in the pattern of the above-mentioned reflexes.

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ONCOLOGY

A CYTOCHEMICAL INVESTIGATION OF THE NUCLEIC ACIDS, PROTEINS AND POLYSACCHARIDES IN MALIGNANT TUMORS OF THE UTERINE CERVIX AND BREAST IN HUMANS

I. A. Shumova

From the Department of General Biology of the Leningrad Institute of Preventive Medicine

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A large number of papers has recently been published, devoted to the explanation of the role of nucleic acids in processes taking place in tumors [1, 5, 9, 10, 11, 12, 15]. It must be pointed out, however, that experimental investigations predominate among this research.

After the work of Warburg [16], great importance began to be attached to polysaccharides in the phenomena of malignant change. There have been few cytochemical investigations, however, devoted to their study [8, 13, 14].

In the present work, particular attention has been directed to the study of nucleic acids, proteins and also polysaccharides in cancer cells, and to the comparison of the patterns observed with the normal. At the same time an attempt was made to establish signs as criteria in the early diagnosis of malignant tissue.

EXPERIMENTAL METHOD

In this research we used clinical material (biopsy specimens, organs removed at operation) from patients with carcinoma of the breast and uterine cervix. As controls we used tissues from these organs taken from the cadavers of persons who had died from various causes (but not from malignant disease), or removed in the course of plastic operations.

Fragments of the organs were fixed in Carnoy's mixture, in alcohol with formalin and also in 10% neutral formalin, and embedded in paraffin wax.

Desoxyribonucleic acid (DNA) was revealed by Feulgen's stain, ribonucleic acid (RNA) by staining with methyl green and pyronin by Unna's method. In order to remove the nucleic acids, sections were treated with a 5% solution of trichloracetic acid (90°, 15 minutes), and to dissolve the RNA we used hydrolysis in normal HCl (60°, 6 minutes).

The protein composition was determined on the basis of staining the preparations with fast green at pH = = 2.2 (total protein) and pH = 8.0 (proteins of the histone type). Polysaccharides were stained by the Shabadash - Hotchkiss method. Controls were set up with ptyalin (60 minutes at 37°) and with distilled water (60 minutes at 37°).

Material was used from more than 100 patients, including 64 with carcinoma of the uterine cervix and breast. In selecting the material, attention was paid to the clinical and pathologicoanatomical data on each patient.

The material investigated included the main clinical stages of malignant disease. The microscopic picture also included the main forms of squamous-cell carcinoma of the uterine cervix (carcinoma "in situ", squamous-cell carcinoma without marked keratinization, keratinizing and undifferentiated carcinoma) and of carcinoma of the breast (adenocarcinoma, scirrhous, encephaloid and solid carcinoma).

EXPERIMENTAL RESULTS

In the normal stratified squamous epithelium of the vaginal part of the uterine cervix, DNA is mainly contained in the nuclei of the basal layer; only insignificant amounts of DNA are present in the cells of the remaining layers. The epithelium of the terminal divisions of the breast also have a comparatively low DNA content.

Weak pyroninophilia was found in the cytoplasm of the cells of the basal layer of epithelium of the vaginal part of the cervix; in the glandular epithelium of the breast this was not observed in the majority of cases; the RNA content increased the nearer the layer to the stratum corneum. It was also present in the nucleoli, in which there is normally only a small amount or none at all. These findings were in agreement with those in the literature [7].

The intensity of the staining for proteins of the histone type and its localization in normal cells corresponded to the picture observed when Feulgen's method for DNA was used.

The epithelium of the vaginal part of the uterine cervix is normally rich in polysaccharides, which, in the form of granules, fill the cytoplasm of all the cells except those of the basal layer. The cells of the terminal divisions of the breast are without polysaccharides. These are found in the form of large granules in the lumen of the alveoli. The connective tissue stroma of the uterine cervix and breast gives a strongly positive diffuse periodic acid – Schiff reaction.

Since treatment with ptyalin did not lead to weakening of the intensity of staining, it may be considered that this particular polysaccharide was not glycogen, and it must therefore be regarded as a mucopolysaccharide.

Our investigations confirmed the data in the literature on the significantly greater nucleic acid content of tumors by comparison with normal tissue [2, 3, 4, 5, 11].

At the present time two types of cells are distinguished in malignant tumors. The cells of the first type (A) are situated in the growing, peripheral part of the tumor; cells of the second type (B) enter into the composition of the inner zone, in which the processes of protein synthesis and growth are very greatly reduced in intensity, and in which mitoses are absent [9].

Type A cells are characterized by a high DNA content in the nucleus and a high RNA content in the cytoplasm and nucleoli. Mitoses are common in these cells. The cytoplasm of the type B cells is poor in RNA, and their nuclei are less rich in chromatin than those of the type A cells; their nucleoli are larger, 3-4 in number, and mitoses are absent. These cells are in a transitional state, after which senescence and degradation begin [5].

Our investigations confirmed the presence of these two types of cells, but the material which we examined led to the necessity of distinguishing a third type of cells which were found in the malignant tumors of the uterine cervix and breast. These cells were characterized by a high DNA content in their nuclei, whereas the pyroninophilia of their cytoplasm was very weak, and the RNA content of their nucleoli was low or it was absent altogether (Fig. 1, a). The nuclei of these cells were often polymorphic; they gave a strong Feulgen reaction and stained intensively with fast green for proteins of the histone type. Mitoses were found among them.

Cells of this type were predominant in such forms of cancer as carcinoma "in situ", which is a malignant process of the epithelium of the uterine cervix that has not yet penetrated into neighboring tissues.

The majority of workers regard it as the initial stage of carcinoma [6]. These cells were also observed at the periphery of the alveoli of the carcinoma.

According to our observations, cells of this type were characteristic of early stages of development of carcinoma, and we therefore referred to them as type A_1.

Analysis of the different forms of carcinoma of the uterine cervix and breast was made in respect to the three types of cell (A_1, A and B) which have been enumerated. A definite trend was established in the

quantitative proportions of these cell types in the various forms of carcinoma of both the cervix and breast.

In carcinoma "in situ" of the uterine cervix, for example, cells of types A_1 and A predominated, especially the first. In squamous-cell carcinoma, cells of types A_1 and A again predominated, but the second of these was more common. Alongside these, type B cells were also present. In the undifferentiated carcinoma, cells of types A and B were most common and there were few type A_1 cells (Fig. 1,b).

Fig. 1. a)Carcinoma "in situ" of the uterine cervix. Atypical stratified epithelium is infiltrating the submucosal tissue along the course of the glands: type A_1 cells — the cell nuclei are rich in DNA, pyroninophilia of the cytoplasm and nucleolus is feebly shown or absent (1); type A cells — the cell nuclei contain DNA, the cytoplasm is rich in RNA, nucleoli are seen (2). Fixing agent — Carnoy's fluid. Stained by Unna's method; b) undifferentiated carcinoma of the uterine cervix. The cancer cells form continuous fields, type A cells (1); type B cells — considerable decrease in the DNA content of the nucleus and the RNA content of the cytoplasm, large nucleoli, rich in RNA and possibly several in number (2); type A_1 cells (3); mitoses (4). Fixing agent, Carnoy's fluid. Stained by Unna's method.

An identical phenomenon was also observed in the material from carcinoma of the breast: in adenocarcinoma type A_1 and type A cells were predominant, and in scirrhous and encephaloid carcinoma cells of types A_1, A and B were found, type A cells being predominant in the scirrhous form. In solid carcinoma type A and type B cells were most often found, and type A_1 cells less so.

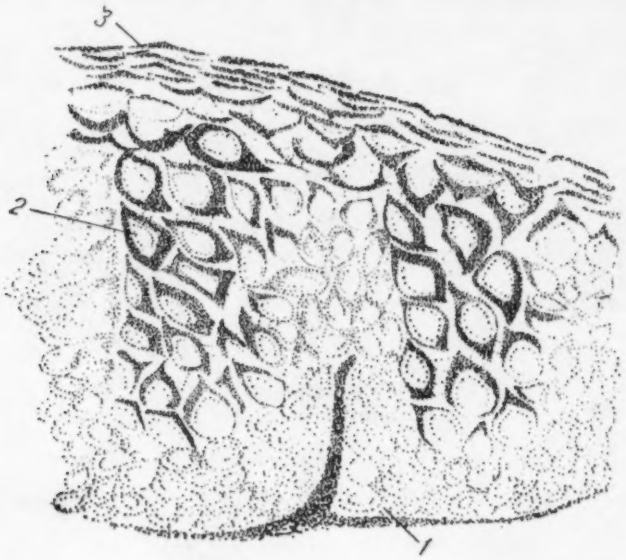

Fig. 2. Carcinoma "in situ" of the uterine cervix. Intra-epithelial infiltration of carcinoma cells: the cytoplasm of the cells contains no polysaccharide or an insignificant amount of it (1); the intervals between the carcinomatous infiltrations are filled with epithelial cells, the cytoplasm of which is rich in polysaccharides; intercellular bridges are seen (2), stratum corneum (3). Fixing agent, Carnoy's fluid. Stained by the Shabadash – Hotchkiss method.

The initial processes of malignant change were thus characterized by the accumulation of DNA in the cell nuclei. Type A_1 cells may be used as one of the early signs of malignant tissue. As the process of malignant change developed and the degree of malignancy increased, there was a growth of RNA synthesis in the tumor, as was shown by the increase in the number of type A cells.

In the dying cancer cells, destruction of DNA and RNA was observed; the latter was preserved mainly in the nucleolus, so that an increased number of type B cells was seen.

When stained by the Shabadash – Hotchkiss method, the cancer cells were found to be poor in or completely devoid of polysaccharides. A definite quantitative relationship was found to exist, opposite to that which was observed in respect to the nucleic acids: the peripheral cells of the tumor were poorest of all in polysaccharides, and their content in the central cells grew.

In carcinoma "in situ" of the uterine cervix the intra-epithelium infiltrations of carcinoma cells contained no polysaccharides, but the intervals between them were filled with epithelial cells, rich in polysaccharides (Fig. 2).

Thus at an early stage of malignant change, during the formation of foci of carcinoma, the surrounding tissue responded by a sharp rise in the content of polysaccharides in the cytoplasm. As the process developed, the polysaccharides were probably used up and nucleic acids were accumulated. In degenerating areas of the tumor, destruction of the nucleic acids took place and polysaccharides were once again accumulated.

SUMMARY

The author determined the content of nucleic acids, proteins and polysaccharides in the cells of cancer tumors of the human breast gland and uterine cervix by the cytochemical method. The data obtained were compared with the normal values. Biopsy material and organs removed during operations were examined. Cancer cells differ from the normal ones by a much higher nucleic acid and low polysaccharide contents.

Analysis of various cancer forms demonstrated a number of regularities, which may be an aid for the early diagnosis of cancer tumors in the future.

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THE EFFECT OF VARIOUS DOSES OF PENETRATING RADIATION ON THE ANTIGENIC AND BIOLOGICAL PROPERTIES OF BROWN-PEARCE CARCINOMA IN EXPERIMENTS IN VITRO

COMMUNICATION I. CHANGES IN THE ANTIGENIC PROPERTIES

I. N. Maiskii, G. V. Suvorova and P. P. Filatov

From the Laboratory of Noninfectious Immunology of the Insititute of Experimental Biology (Director — Prof. I. N. Maiskii) of the AMN SSSR, Moscow

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There has recently appeared a comparatively large number of papers devoted to changes in the biochemical properties of cells and tissues under the influence of penetrating radiation [1, 2, 5, 6, 7, 15, 16, 17].

In a number of papers [4, 10, 11, 12, 13, 14] changes have been shown in the antigenic structure of normal tissues under the influence of x-rays.

Arising from the hypothesis put forward by N. N. Zhukov-Verezhnikov, I. N. Maiskii and G. S. Gostev [3] on the character of the antigenic variation of tumor cells under the influence of ionizing radiation, we carried out a series of investigations of the action of x-rays on the antigenic properties of tumor tissues.

In our previous communications [8, 9] we have pointed out changes in the antigenic and biological properties of the cells of a mouse cancer (the ascitic form of Ehrlich's carcinoma) under the influence of x-rays in vitro.

The aim of the present research was to study the variation of the antigenic composition of the cells of the Brown-Pearce rabbit carcinoma under the influence of penetrating radiation (in particular of x-rays) and the relationship between these changes and the dose of irradiation.

EXPERIMENTAL METHOD AND RESULTS

The investigation was carried out on a 20% suspension of Brown-Pearce carcinoma cells, irradiated by x-rays in vitro doses of 2000, 5000, 10,000 and 20,000 r.

The suspension of cells was prepared as follows: in a rabbit affected by a Brown-Pearce tumor, tissue was taken from tumor nodules showing no signs of necrosis, weighed, placed in a sterile Petri dish and cut into small pieces with scissors to obtain a homogeneous mass, physiological saline being gradually added to the dish in proportion of 1:5. The 20% suspension of Brown-Pearce cells thus obtained was poured in volumes of 2.8 ml into glass bottles with a basal diameter of 3.5 cm, and irradiated with x-rays in the doses given above.

Irradiation was given by means of an RUT-60-20 (RUM-7) apparatus. The conditions of irradiation in all the experiments were the following: for a dose of 20,000 r, voltage 60 kv, current 20 ma, filter 0.1 Al, focal distance 75 mm, time of irradiation 36 seconds. For the remaining doses the conditions of irradiation were altered only in respect to the current strength and the time of exposure. For a dose of 10,000 r, for instance, the current was 10 ma and the exposure 37 seconds, and for a dose of 5000 r the current was 5 ma and the exposure 15 seconds.

TABLE 1

Results of the Anaphylaxis with Desensitization Reaction

Sensitization. Antigen — Brown-Pearce carcinoma cells irradiated with 5000 r	Desensitization. Antigen — untreated Brown-Pearce carcinoma cells						Assaulting injection. Antigen — Brown-Pearce carcinoma cells irradiated with 5000 r	
	1st injection		2nd injection		3rd injection			
dose	dose	reaction	dose	reaction	dose	reaction	dose	reaction
0.5	0.2	++	0.35	+++	0.5	—	0.5	++
0.5	0.2	+	0.35	—	0.5	—	0.5	+
0.5	0.2	++	0.35	±	0.5	—	0.5	++
0.5	0.2	+++	0.35	+	0.5	—	0.5	+++
0.5	0.2	++	0.35	++++				

Legend: —) no reaction; ±) a single scratching of the snout, and sneezing; +) repeated scratching of the snout and sneezing; + +) the same, with cough, instability; + + +) the same, with convulsions and incontinence of urine and feces; + + + +) the same, and death.

Note. The table does not include guinea pigs used for control of the toxicity of the preparation, nor also the control group of animals sensitized and desensitized with untreated tumor cells, since the former demonstrated that the doses used for the assaulting injection were nontoxic, and the latter gave no reaction to the assaulting injection in consequence of their complete desensitization with the untreated tumor tissue.

The viability of the irradiated cells was tested by intratesticular inoculation of rabbits.

The changes in the antigenic properties of the tumor cells under the influence of x-rays were studied by means of the anaphylaxis with desensitization reaction. Sensitization was carried out subcutaneously, and desensitization and the assaulting injection were given by intracardial injection. The interval between sensitization and desensitization was 22 days, and the interval between repeated intracardial injections was 2 hours.

In order to answer the question whether it was possible for the antigenic properties of the Brown-Pearce carcinoma cells to be changed under the influence of ionizing radiation, guinea pigs of the experimental group were sensitized by tissue of a Brown-Pearce tumor irradiated with 5000 r and densensitized by untreated carcinoma tissue. After testing for complete desensitization, as an assaulting injection we used tissue of a Brown-Pearce tumor irradiated with 5000 r. As a control of the experimental group we used guinea pigs sensitized and desensitized by unirradiated carcinoma tissue, and also guinea pigs on which the toxicity of the dose used for the assaulting injection was tested.

In Table 1 are given the results obtained in the first series of experiments.

As may be seen from the results in Table 1, the guinea pigs sensitized with irradiated tumor tissue could not be completely desensitized by untreated Brown-Pearce carcinoma tissue. All the guinea pigs reacted to the assaulting injection of irradiated tumor tissue by reactions from + to + + +.

Under the influence of irradiation with x-rays in vitro, changes therefore took place in the antigenic structure of Brown-Pearce carcinoma cells.

In order to answer the next question — whether the changes in the antigenic composition of the tumor cells depended on the dose of irradiation — 3 experimental groups of guinea pigs were used, which were sensitized with tumor tissue irradiated with 10,000 and 20,000 r, and desensitized with tissue irradiated with 2000 and 10 000 r.

After testing for completeness of desensitization, as assaulting injection we used tissue irradiated with the same dose of x-rays as that used for irradiating the tissues during sensitization. Thus, the first experimental

group of guinea pigs was sensitized with tissue irradiated with 10,000 r, and desensitized with tissue irradiated with 2000 r. As assaulting injection, tissue irradiated with 10,000 r was used. The second experimental group of guinea pigs was sensitized with tissue irradiated with 20,000 r and desensitized with tissue irradiated with 2000 r, and as assaulting injection, tissue irradiated with 20,000 r was used. The guinea pigs of the third experimental group were sensitized with tumor tissue also irradiated with 20,000 r, but were densitized with tissue

TABLE 2

Results of the Anaphylaxis with Desensitization Reaction with Antigens from Brown-Pearce Carcinoma Tissue Irradiated with Different Doses of X-Rays

Experimental group	Sensitization		Desensitization					Assaulting injection	
	antigen	dose	antigen	1st injection 0.2	2nd injection 0.35	3rd injection 0.5	4th injection 0.5	antigen	reaction
1	Brown-Pearce carcinoma tissue, irradiated with 10,000 r	0.5 0.5 0.5 0.5	Brown-Pearce carcinoma tissue, irradiated with 2000 r	++++ +++ ++ +++	++ — ++	+ — ±	— — —	Brown-Pearce carcinoma tissue, irradiated with 10,000 r	+++ ++ ++
2	Brown-Pearce carcinoma tissue, irradiated with 20,000 r	0.5 0.5 0.5 0.5		++ +++ + +++	++ ++ ++ —	+ — — —	— — 	Brown-Pearce carcinoma tissue, irradiated with 20,000 r	+ ++++ ++++ +++
3		0.5 0.5 0.5 0.5	Brown-Pearce carcinoma tissue, irradiated with 10,000 r	++ +++ + +	+ ± — —	— — — —			± ++ ++ +

Legend as in Table 1.

Note. The table does not include the above-mentioned control groups of guinea pigs, since during sensitization and desensitization of the animals with tissue irradiated with the same dose of x-rays, complete desensitization of the guinea pigs was obtained, and injection of the animals with a suspension of tumor cells irradiated with 10,000 and 20,000 r as assaulting injection was found to be nontoxic.

irradiated with 10,000 r, and for the assaulting injection tissue was used which had been irradiated with 20,000 r.

As controls for the experimental group of animals in the second series of experiments, guinea pigs were used which had been sensitized and desensitized by tumor tissue irradiated with the same dose of x-rays, and also guinea pigs on which the toxicity of tumor cells irradiated with 10,000 and 20,000 r had been tested.

The results obtained in the second series of experimenta are shown in Table 2.

The above-mentioned control groups of guinea pigs are not included in Table 2, for in the course of sensitization and desensitization of the animals with tissue irradiated with the same dose of x-rays, complete desensitization of the guinea pigs was obtained, and injection of the animals with a suspension of tumor cells irradiated with 10,000 and 20,000 r as assaulting injection was found to be nontoxic.

It can be seen from the results in Table 2 that during sensitization of guinea pigs with tissue irradiated with 10,000 r complete desensitization did not take place when they were given injections of cells irradiated with 2000 r, just as in the case of sensitization of the animals with cells irradiated with 20,000 r, it was impossible to obtain complete desensitization by injecting tissue irradiated with 2000 r or 10,000 r.

Under these circumstances it must be pointed out that on the average the strength of the reaction of the guinea pigs to the assaulting injection was proportional to the dose of x-rays used for irradiation of the tissue in sensitization and desensitization.

As may be seen from the results in Table 2, the most pronounced reaction to the assaulting injection was observed in the guinea pigs of the second group, sensitized with tumor cells irradiated with 20,000 r, and desensitized with tissue exposed to the action of 2000 r.

We observed the least pronounced reaction in the guinea pigs of the third group, sensitized with a suspension of cells irradiated with 20,000 r and desensitized with similar material irradiated with 10,000 r.

Hence it followed that different doses of ionizing radiation clearly caused unidentical changes in the antigenic composition of the Brown-Pearce tumor.

The results obtained in the first and second series of experiments led to the conclusion that penetrating radiation, and in particular x-rays, cause a change in the antigenic composition of the Brown-Pearce carcinoma of rabbits.

These changes in the antigenic structure of the tumor tissue varied in relation to the dose of irradiation, and moreover the greater the difference between the doses of irradiation, the greater the change appeared in the antigenic structure of the irradiated tumor tissue.

SUMMARY

The work deals with the study of the effect of penetrating radiation (particularly of x-rays) on the antigenic structure of the Brown-Pearce carcinoma cells as well as with the establishment of a relationship between the irradiation doses and the changes in the antigenic structure of the tumor tissue.

The data obtained show that x-rays produce a change in the antigenic structure of the Brown-Pearce carcinoma. It varies depending on the irradiation dose (2000 r, 5000 r, 10,000 r and 20,000 r). The higher the irradiation dose the greater the changes of the antigenic structure of the irradiated tumor tissue.

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THE FORMATION OF IMMUNE ANTIBODIES IN RABBITS DURING THE GROWTH OF A BROWN-PEARCE TUMOR

G. P. Airapet'yan

From the Laboratory of Noninfectious Immunology (Head – Prof. L. N. Maiskii) of the Institute of Experimental Biology (Director – Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received October 24, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

The research of A. A. Bogomolets and his co-workers [1] revealed the important role of the reticulo-endothelial system in the resistance of the body to malignant disease.

One of the indices of the state of the defensive forces of the body is its power of formation of antibodies.

The findings in relation to the study of immunogenesis in the presence of malignant neoplasms are very contradictory. I. Kirichinskaya and M. Sirotina [2] studied the sera of rabbits with an implanted Brown-Pearce tumor, immunized with sheep's red cells, and found a lower titer of hemolysins by comparison with the sera of immunized normal rabbits. Certain authors have conducted a clinical investigation of immunogenesis in patients with malignant tumors. As antigens for immunization, bacteria, viruses and toxins were used. Various methods were used, and the titers of the variety of heterophilic antibodies – complement-fixing, agglutinins and precipitins – were determined. The investigations of Parfent'ev, Clifton and Duran-Reynals [7], and Southam, Goldsmith and Burchenal [8] showed that in patients with malignant neoplasms, production of antibodies was feebler than in a group of persons not suffering from tumors. On the other hand, Logan [6], Larson and Tomlinson [4] and Leskowitz, Phillipino et al. [5] found no difference in antibody formation between cancer patients and healthy persons. In some cases the authors did not take into consideration the character of the antigenic stimulus, and immunized with a mixture of different antigens, and so on, which is very important in immunogenesis [3]. The discrepancies in the findings on this problem led to the necessity for further research.

The aim of our work was to study immunogenesis in rabbits with implanted Brown-Pearce tumors by comparison with that in healthy animals, when immunized with a heterogeneous malignant antigen – the ascitic form of Ehrlich's adenocarcinoma of mice, and a homologous antigen – Brown-Pearce tumor.

EXPERIMENTAL METHOD

In the first series of experiments 16 male rabbits of the chinchilla variety were divided into 2 equal groups. The eight animals of the first group were given intratesticular inoculations of a 20% suspension of Brown-Pearce tumor cells. The second group, consisting of 8 healthy rabbits, were used as controls. Sere were obtained from all the rabbits and the preliminary background titer was determined in the complement fixation reaction with antigens from ascitic cells of Ehrlich's adenocarcinoma, Brown-Pearce tumor and the liver and spleen of normal mice and rabbits. On the 7th day after inoculation, when development of the carcinoma could be detected clinically, immunization of the first and second (control) groups began. As antigen we used a saline extract of ascitic cells of Ehrlich's adenocarcinoma in a dilution of 1:20. Immunization was carried out by injection of 1 cm^3 of antigen intravenously on 3 successive days.

The short cycle of immunization was due to the fact that the animals might die from the carcinoma before the immune sera had been obtained from them. On the 7th day, after the completion of immunization, blood was taken from the rabbits, and subsequently blood was taken every 5 days. In the sera which we obtained we determined the antibody titer in the complement fixation reaction at 37° and observed its change with time. In the animals dying with signs of malignant cachexia, we studied the macroscopic picture, post mortem.

In the second series of experiments we used 12 rabbits, 6 in each group. The conditions of the experiment were analogous to those described, except that immunization was done with a saline extract of Brown-Pearce tumor in a dilution of 1:20, i.e., with a homologous antigen.

EXPERIMENTAL RESULTS

In the table are given the results of the first series of experiments. Here are shown the antibody titers of the immune sera of rabbits with carcinoma and of healthy rabbits, as revealed by the complement fixation reaction with an identical antigen, with a saline extract of ascitic cells of Ehrlich's adenocarcinoma in mice. The antibody titers were studied on the 17th, 22nd, 27th and 32nd days after implantation of the tumor. The serum dilutions used were from 1:50 to 1:400. As an additional control, in the complement fixation reaction, in addition to antigen against which the immune sera were obtained, we used additional antigens prepared from tissues of normal organs of mice — the spleen and liver — and also antigen from Brown-Pearce tumor tissue. In the table, however, for the purpose of simplification, we do not give the results of these control experiments. In the right hand column of the table are indicated the time of death of the animals suffering from carcinoma.

As can be seen from the table, the titers of immune antibodies in the group of rabbits suffering from carcinoma were mainly much lower than those in the control group, consisting of healthy, immune animals. A reaction of + + + and + + + + was observed in 4 of the 8 rabbits with carcinoma, most often in a dilution of 1:50. The exception was rabbit No. 2204, which, in spite of its rapid death (on the 28th day), showed a quite high antibody titer, giving fixation of + + + in dilution of 1:200.

At postmortem examination metastases were found in the spinal cord with resulting paralysis, whereas the parenchymatous organs were free from metastases. This may probably explain the comparatively high antibody titer in the sera, in spite of the animal's rapid death. The four remaining rabbits of this group, even in dilution of 1:50, fixed complement only to the extent of + and + +. In the control group the immune sera reacted to + + + and + + + + in dilution of 1:200 and in some cases of 1:400.

Thus a fall was clearly seen in the power of antibody formation to this particular antigen by rabbits suffering from carcinoma. We were unable to establish any regular relationship in the trend of antibody formation at the different stages of the malignant disease, since the antibody titers were roughly the same over the period of 20 days during which blood was taken.

In the second series of experiments immunization was carried out with a saline extract of a Brown-Pearce tumor. In order to determine the titers of immune antibodies lower dilutions of sera were used — from 1:20 to 1:160.

Furthermore, as an additional control in this series we used an immune goat serum to Brown-Pearce tumor, and also sera of rabbits inoculated with Brown-Pearce tumors at the same times but not subjected to immunization with a saline extract of this tumor.

The complement fixation reaction was performed both with antigen from the Brown-Pearce tumor and with antigens from normal tissues — spleen and liver of rabbits.

In the experimental and control groups, immunization with homologous tumor antigen did not produce antibody formation. The exceptions were 3 rabbits (Nos. 459, 606 and 893), in which after the formation of metastases (in two — metastases in the eyes, in the third — in the internal organs) the malignant process terminated spontaneously and the tumor was absorbed. In these animals antibodies were detected in dilutions of 1:20 to 1:80 to the extent of + + + and + + + +. We did not, however, attribute the presence of these antibodies to injected antigen, but we regarded their appearance as due to the absorption of the tumor.

The investigations carried out demonstrated that the formation of immune antibodies in rabbits in response to the injection of heterologous and homologous tumor antigens depended not only on the state of the animal but also on the character of the antigenic stimulus.

Titers of Complement-Fixing Antibodies in the Blood of Rabbits with Carcinoma and Healthy Rabbits after Their Immunization with Heterologous Antigen

Group of rabbits	Rabbit No.	Day after inoculation on which blood was taken: 17th — dilutions of sera 1:50	1:100	1:200	1:400	22nd — 1:50	1:100	1:200	1:400	27th — 1:50	1:100	1:200	1:400	32nd — 1:50	1:100	1:200	1:400	Day of death of rabbit
With implanted Brown-Pearce tumor	2603	++	+	−	−	++	+	−	−	−+	−	−	−	++	+	−	−	40-th
	1061	+++	++	+	−	+−−	++	+	−	−−−	+−	−	−	++	+	−	−	35 th
	3780	++++	+++	+	−	−+−	++	+	−	−−	−	−	−	+	−	−	−	34 th
	1883	+++	++	+	−	−+	+	−−	−	−−	−	−	−					29 th
	2204	++++	+++	+++	++	++++	+++	++	−+	++++	+++	−−	−					28 th
	3368	++	+	−	−	+++	++	+	−									23 rd
	2045	++	+	−	−	++	−	−	−									23 rd
	3612	+	−	−	−													20 th
Control	3121	++++	++++	+++	++	++++	++++	+++	++	++++	+++	+++	++	++++	++++	+++	++	
	3535	++++	++++	+++	++	++++	+++	++	++	++++	++++	+++	++	++++	+++	+++	++	Rabbits survived
	2537	++++	++++	+++	++	++++	++++	+++	+++	++++	++++	+++	++	++++	+++	++	+	
	3482	++++	++++	+++	++	++++	++++	+++	++	++++	++++	+++	++	++++	++++	+++	++	
	2862	++++	++++	+++	+	++++	+++	++	+	++++	+++	++	+	++++	++++	+++	++	
	2445	++++	+++	+++	++	+++	+++	++	+	++++	+++	++	++	++++	+++	++	++	
	3811	++++	+++	+++	++	++++	++++	+++	++	++++	++++	+++	++	++++	+++	++	+	
	3315	++++	++++	+++	+++	++++	++++	+++	+++	++++	++++	+++	++	++++	++++	+++	+++	

At the same time these results showed that immunogenesis in rabbits suffering from carcinoma was considerably suppressed by comparison with healthy animals.

SUMMARY

Experiments were conducted on healthy rabbits and on those suffering from cancer. The author undertook a comparative study of the antibody production resulting from administration of heterologous antigen (water-salt extract of the ascitic cells of the Ehrlich adenocarcinoma),and of homologous antigen (water-salt extract of the Brown-Pearce tumor). In immunization with a heterologous tumor antigen immunogenesis was depressed in the affected animals in comparison with the control ones. As to immunization with a homologous tumor antigen, it caused no antibody formation either in the experimental or in the control group of animals.

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ANTIGENIC PROPERTIES OF THE RED CELLS IN LEUKEMIA

COMMUNICATION III. SPECIFIC INHIBITION OF PRECIPITATION REACTION IN AGAR ON A GLASS SLIDE USING RED CELLS FROM LEUKEMIA PATIENTS

D. M. Levina, F. Lacour and V. A. Parnes

From the Division of Immunology and Malignant Tumors (Head – Active Member AMN SSSR L. A. Zil'ber) of the N. F. Gamaleya Institute of Epidemiology and Microbiology (Director – Prof. S. N. Muromtsev) of the AMN SSSR and the Institute Gustave Roussy (Director – Dr. P. Denoix)*, France.

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By means of Ouchterlony's specific inhibition of precipitation reaction in agar [4, 5], it has been possible to detect a difference in the antigenic properties of the red cells of healthy donors and of patients with acute leukemia, chronic lymphatic and myeloid leukemias and reticulosis [1-3].

The possibility of differentiation of the red cells from patients with leukemia and those from healthy donors has opened prospects for the use of this method as an accessory diagnostic test.

In order to simplify the technique and to obtain more rapid results, we decided to use the precipitation reaction in agar on a glass slide.

EXPERIMENTAL METHOD

The test was performed on ordinary glass slides, thoroughly freed from fat, with ground ends on which the number of the experiment was marked.

For the test we used 1% transparent agar, which was first freed from contaminants by precipitation with calcium chloride, filtration and washing for 72 hours in tap water.

One drop of molten sterile agar, with merthiolate preservative (1:10,000) was placed on the edge of a warm glass slide. A thin film was made, like a blood film. After the film had dried, a second layer of agar was applied, 1-2 ml in volume.

Next, by the use of a special metal drill, connected by a rubber tube to a glass end-piece, shallow holes of equal diameter were drilled in the coagulated agar. The drilling was done carefully in order to avoid damaging the lower layer of agar and the side walls of the holes. The agar fragments produced during drilling were removed by suction through the rubber tube.

The arrangement of the holes on the slide may be seen in Fig. 1.

The test was carried out in two stages: the first: into the central hole was poured the antigen from the healthy donors' red cells in a titrated dose; the second: into the same hole, after 4-13 hours (after complete diffusion of the antigen), was poured 0.01 ml of antiserum obtained after immunization of rabbits with red cells

* Dr. P. Denoix is director of the Clinical Center of the Institute Gustave Roussy, Villejuif (Seine), France.

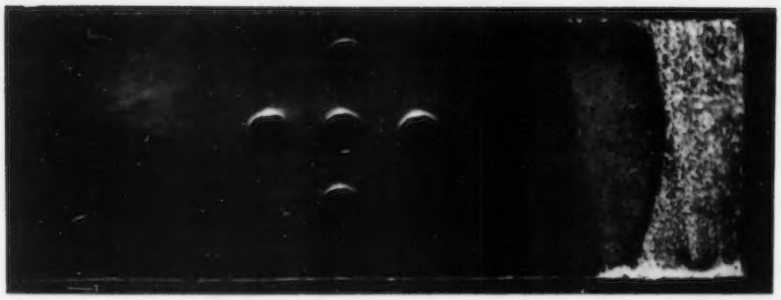

Fig. 1. Glass slide with holes drilled in the layer of agar.

from patients with leukemia, and into the peripheral holes were poured antigens from red cells of healthy donors and patients, also in volume of 0.01 ml.

All the antigen preparations were preliminarily standardized for protein content per ml. The dose of each preparation in an experiment did not usually exceed 0.1 mg of protein.

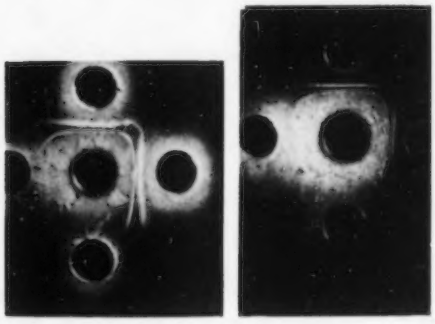

Fig. 2. Specific inhibition of precipitation reaction in agar with antiserum to red cells from patients with leukemia. In the agar are antigens from the red cells of healthy donors. In the holes: in the center – antiserum to red cells from patients with leukemia; in the top and right: antigens from red cells of a patient with chronic myeloid leukemia; antigens from the red cells of a patient with acute leukemia; in the bottom and left: antigens from the red cells of healthy donors of the same blood group.

Each antigen was tested on not less than 4 glass slides. The patients' red cells were compared with the healthy donors' red cells of the same blood group by the ABO and Rh systems. The slides were left at room temperature in a humid chamber. The results were read after 4-18 hours.

In all,34 patients with leukemia were tested (8 with acute leukemia, 6 with chronic lymphatic leukemia, 12 with chronic myeloid leukemia, 3 with aleukemic leukemia and 5 with reticulosis) as well as 36 healthy donors. Each specimen of blood was submitted to not less than 4 tests.

EXPERIMENTAL RESULTS

The antigenic spectrum of the red cells from both normal and leukemic patients was complex, and therefore in the differentiation of the antigenic properties of patients and healthy donors we recommend the use of the specific inhibition of precipitation reaction in agar. This reaction differs from the ordinary precipitation reaction agar, introduced by Ouchterlony [5], by the fact that antigen from the red cells of normal donors is preliminarily introduced into the agar.

With a properly selected dose, this permits total suppression of formation of precipitation lines in the agar during the reaction between the antiserum and the red cells of healthy donors. If under these circumstances the antiserum preserves its power to give precipitation lines with the red cells of patients with leukemia, the result is regarded as positive (Fig. 2, a and b).

In the table are shown the results of the specific inhibition of precipitation reaction on a glass slide.

As can be seen from the results in the table, in 119 of 138 tests (i.e., 86%) the red cells of the patients were clearly distinguished in their antigenic properties from the red cells of the healthy donors. The method of specific inhibition of precipitation on a glass slide may thus be used to differentiate the red cells of patients with leukemia and of healthy donors of the same blood group (by the ABO and Rh systems). The method is simple, requires small quantities of ingredients and gives quick results.

Specific Inhibition of Precipitation Reaction in Agar on a Glass Slide, with Antigens from the Red Cells of Patients with Leukemia and Healthy Donors

Tests	Patients with a diagnosis of						Healthy donors
	acute leukemia	chronic lymphatic leukemia	chronic myeloid leukemia	aleukemic leukemia	reticu-losis	total	
Total number of tests	33	24	48	12	21	138	130
Number of positive tests	27	21	42	12	17	119	0
Percentage of positive tests	82	90	87	100	81	86	0

The character of the antigenic differences which we found is being studied.*

SUMMARY

The authors used the reaction of specific inhibition in agar on a slide by employing the antiserum for the red cells with high precipitating titer of patients suffering from leukemia. In 86% of the cases it was possible to reveal a difference in the antigenic properties of red cells obtained from the patients suffering from leukemia (acute leukemia, chronic myelo- and lympholeukemia, reticulosis and aleukemic leukemia) and in those of healthy donors with the same blood group (according to the ABO and Rh systems).

The method of specific inhibition of precipitation in agar on a slide enables one to quickly differentiate by their antigenic properties the red cells of healthy donors and those of the patients suffering from leukemia.

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** See English translation.

THE EFFECT OF ANTITUMOR SERUM ON THE INTENSITY OF CELL DIVISION IN TUMORS

S. V. Sukhorukikh

From the Laboratory of Noninfectious Immunology (Head — Prof. I. N. Maiskii) of the Institute of Experimental Biology (Director — Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received November 20, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

Several workers [1, 3, 6, 7, 8, 11, 12] have shown that antitumor sera lengthen the latent period of development of cancer, and retard the growth and progress of tumors and formation of metastases.

It has also been shown [2, 9, 10] that antitumor serum, when injected into experimental animals with developing tumors (Ehrlich's adenocarcinoma) has a depressing action of the mitotic activity of tumor cells in comparison with sera of different specificity.

As a rule, however, the antitumor sera used experimentally contained, besides antibodies against the tumor, antibodies against normal tissues.

The aim of the present work was to compare the action of an adsorbed antitumor serum, containing mainly antibodies against the tumor, with that of ordinary unadsorbed antitumor serum on the intensity of division of the tumor cells in a subcutaneous Ehrlich's adenocarcinoma.

As a control we used the serum of a normal, nonimmune rabbit and sera against the liver and spleen of a healthy mouse. The latter appeared desirable in view of the antigenic closeness of the spleen and the tumor [4]. The mitotic activity was studied, besides in the tumor, in the corneal epithelium of the same experimental animals in order to ascertain the effect of sera of different specificity on it.

EXPERIMENTAL METHOD

The sera used in the experiments were obtained by immunization of rabbits of the chinchilla variety with saline extracts of the ascitic cells of an Ehrlich's adenocarcinoma and of the liver and spleen of a healthy mouse.

The rabbits were immunized by means of 6 intravenous injections at intervals of 3 days.

The sera obtained were tested by means of the complement fixation reaction at a temperature of 37° for the height of the titer of the antibodies contained in them and for their specificity. The experiments comprised series I and II.

The following sera were used in the experiments in series I:

1). Adsorbed antitumor serum which reacted with tumor antigen in a dilution of 1:160 + + +, spleen 1:40 + + and liver 1:40 ±.

2). Unadsorbed antitumor serum with a titer of antitumor antibodies of 1:400 + + +, antispleen 1:200 + + and antiliver 1:100 ±.

3). Antispleen serum with a titer of antispleen antibodies of 1:200 + + +. It reacted with tumor antigen in a dilution of 1:100 + + but did not react with liver antigen in dilutions of 1:100 or higher.

4). Serum of a normal, nonimmune rabbit, containing no antibodies against the antigens tested.

TABLE 1

Mitotic Coefficient (MC) in Mouse Tumors in Series I Experiments after Three Injections of Sera (per 1000)

Mouse No.	Sera				Control (nothing injected)
	absorbed antitumor 1:160 + + +	unadsorbed antitumor 1:400 + + +	antispleen 1:200 + + +	normal rabbit's	
	1st group	2nd group	3rd group	4th group	5th group
1	0,70	4,08	7,97	6,60	8,75
2	4,48	4,17	8,82	7,38	10,90
3	7,36	4,65	10,55	9 06	11,41
4	7,71	4,99	10,93	9,47	11,50
5	7,77	5,36	11,14	10,40	11,50
6	8,22	5,66	11,51	11,60	12,13
7	8 36	7,04	12,23	13,87	18,14
8	Necrosis of tumor	7,43	14,56	18,03	
Mean MC	6,37	5,42	10,96	10,78	12,04

Probability of chance variation of mean values of:

1 and 3 groups $P=0,003$
1 and 4 groups $P=0,027$
1 and 5 groups $P=0,003$
2 and 3 groups $P=\infty$
2 and 4 groups $P=0,002$
2 and 5 groups $P=\infty$

It must be pointed out that the height of the titer of antitumor antibodies in the adsorbed antitumor serum and of antispleen antibodies in the antispleen serum were on roughly the same level.

The following sera were used in the experiments in series II.

1). The adsorbed antitumor serum had identical immunological characteristics with the analogous serum used in series I.

TABLE 2

Mitotic Coefficient (MC) in the Corneas of the Mice in Series I Experiments after Three Injections of Sera (per 1000)

Mouse No.	Sera				Control (nothing injected)
	absorbed antitumor 1:160 + + +	unadsorbed antitumor 1:400 + + +	antispleen 1:200 + + +	normal rabbit's	
	1st group	2nd group	3rd group	4th group	5th group
1	2,64	2,02	2,47	1,38	3,75
2	2,64	2,92	2,63	2,34	3,80
3	3,97	3,45	2,92	3,86	3,94
4	4,21	3,90	3,02	3,87	4,08
5	4,67	4,19	3,43	3,87	4,13
6	4,74	4,19	4,23	4,27	6,92
7	4,90	4,62	4,56	4,56	Preparation spoiled
8	5,41	5,70	5,09	6,01	
Mean MC	4,14	4,12	3,54	3,77	4,44

2). The unadsorbed antitumor serum reacted with tumor antigen in dilution of 1:800 + + +, with spleen 1:400 + + + and liver 1:100 +.

3). The antispleen serum had a titer of antispleen antibodies of 1:800 + + +, antitumor antibodies 1:200 + + and antiliver 1:100 ±.

4). The antiliver serum reacted with homologous antigen in a dilution of 1:800 + +, with spleen antigen in a dilution of 1:200 + +, and did not react at all with tumor antigen in dilutions of 1:100 and higher.

5). The serum of the normal normal nonimmune rabbit contained no antibodies against the antigens tested.

Thus in the experiments of series II, the height of the titer of antitumor antibodies in the unadsorbed antitumor serum, of antispleen antibodies in the antispleen serum and of antiliver antibodies in the antiliver serum were roughly the same.

The sera were adsorbed by the method introduced in Prof. P. N. Kosyakov's laboratory [5], with a mixture of formalinized liver and spleen tissue, but we took whole serum and not serum diluted 1:10 for the adsorption.

In the two series of experiments we used altogether 100 male white mice with an Ehrlich's adenocarcinoma implanted under the skin of the dorsal region. Of these mice, 39 were in series I of the experiments and were divided into 5 groups. One group (7 mice), which received no injections, acted as controls, and the remaining 4 groups (with 8 mice in each) were injected with one of the above-mentioned sera. In series II of the experiments 61 mice were used, and these were divided into 6 similar groups.

The sera were injected three times, on alternate days starting on the 8th day after implantation of the tumor material, subcutaneously in doses of 0.5 ml at some distance from the tumor.

All the experimental animals were killed at the same time, i.e., in the morning 20 hours after the last injection of serum.

The tumor was fixed in Carnoy's fluid. The corneas were fixed in a 5% solution of acetic acid in 70° alcohol. Sections, 8 μ in thickness, were cut from the tumor, and total preparations of the cornea were made. In both cases staining was by Carazzi's hematoxylin.

After counting the mitoses, we calculated the mitotic coefficient (MC) for each test object, which expressed the ratio between the dividing cells and the total number of cells examined (per 1000).

We also calculated the coefficient K (the ratio between the total number of early phases of division and the late phases).

The experimental results were treated statistically. The difference between the values compared was regarded as significant if the value of P was not greater than 0.01.

EXPERIMENTAL RESULTS

The results of the counting of mitotic activity in the mouse tumors of all the experimental groups of the series I experiments showed that the mean percentage of the different phases of division was approximately the same for each group (P ≈ 1-2%; M ≈ 78-83%; A ≈ 3-4%; T ≈ 11-17%). The ratio between the total number of early phases of division and the late phases was also on roughly the same level (K ≈ 4-5), from which it could could be concluded that the tempo of cell division was approximately equal in both the control and the experimental animals. We therefore judged the intensity of cell division mainly by the value of the mitotic coeffient.

The results given in Table 1 show that the values of the mitotic coefficient in the 1st and 2nd experimental groups, in which antitumor (adsorbed and unadsorbed) sera were injected, were the least and were roughly equal (6.37 and 5.42 respectively).

In the remaining groups the value of the mitotic coefficient was much higher (3rd group 10.96, 4th group 10.78, 5th group 12.04). The values of the mitotic coefficient in the groups in which adsorbed and unadsorbed anti-tumor sera were injected were evidence of the identical action of these sera on the intensity of division of the tumor cells.

The results of the counting of mitotic activity in the corneal epithelium of the experimental animals of the series I experiments showed that the mean percentage of different phases of division in the various experimental groups showed slight variations (P ≈ 18-30, M ≈ 34-48, A ≈ 3-5 and T ≈ 23-30). Nevertheless the ratio of the total number of early phases of division to that of the late phases was roughly the same (K ≈ 2-2.6).

The values of the mitotic coefficient are shown in Table 2, from which it can be seen that the mitotic coefficient in all 5 groups varied between narrow limits (4.14, 4.12, 3.54, 3.77 and 4.44). Consequently, no essential change in the mitotic activity could be observed in the corneal epithelium under the influence of sera of different specificity.

The results of the series II experiments were in the main analogous with those obtained in series I. An exception was the group of mice which received an injection of antispleen serum, since the majority of the mice of this group (8 of 11) dies after the injection of this serum with signs of cytotoxic shock. This was evidently due to the excessively high titer of antispleen antibodies (1:800 + + +).

Our findings thus showed that an adsorbed antitumor serum, containing antibodies mainly against the tumor, has the same depressing action on the intensity of division of the tumor cells in a subcutaneous Ehrlich's adenocarcinoma as an unadsorbed anti-tumor serum. The experimental results demonstrate that the inhibitory effect of the antitumor serum on the mitotic activity of the tumor can be ascribed to the presence in the serum of antitumor antibodies.

The antitumor serum (adsorbed or unadsorbed) had no depressing effect in our experiments on cell division in the corneal epithelium of mice developing a subcutaneous adenocarcinoma.

SUMMARY

The effect was compared of adsorbed antitumor serum with mainly tumor antibodies and of the usual nonadsorbed antitumor serum on the intensity of the tumor cell division in the subcutaneous Ehrlich's adenocarcinoma.

The results of experiments staged on 100 male white mice demonstrated that the adsorbed and the nonadsorbed sera exercise an equal inhibitory effect on the mitotic activity of the tumor. The rabbits' serum immunized with liver and spleen tissue of healthy mice as well as the blood serum of normal rabbit exerted no such effect on the tumor. The cited sera effected no changes in the mitotic activity of the corneal epithelium in experimental animals.

Thus, the inhibitory effect of the antitumor serum exerted on the mitotic activity of the tumor, should be ascribed to the presence of antitumor antibodies in it.

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THE ACTION OF HOMOLOGOUS IMMUNE SERA ON THE CELLS OF IMPLANTED TUMORS

G. I. Avdeev

From the Virological Laboratory (Head — V. V. Gorodilova, Consultant — Active Member AMN SSSR L. A. Zil'ber) of the P. A. Gertsen State Oncological Institute (Director — Prof. A. N. Novikov, Scientific Director — Corresponding Member AMN SSSR Prof. A. I. Savitskii), Moscow

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We know of many investigations as a result of which it has been possible to obtain sera possessing a cytotoxic action on tumor cells. For this purpose in the majority of cases heterologous sera were used. In order to explain the mechanisms of antitumor immunity, however, it is much more important to study homologous sera, i.e., sera obtained from animals of the same species as the one bearing the tumor. There are only few investigations of this type (see the detailed survey by R. M. Radzikhovskaya [2]) and fresh information on this problem would be of undoubted interest.

EXPERIMENTAL METHOD

Test sera were prepared by means of immunization of rats with tissue from transplanted rat sarcomas. We tried out several methods of immunization of animals.

Goldfeder [4] irradiated tumor transplantates with definite doses of x-rays which injured the cells but did not cause their death. Being implanted into animals, these transplantates were absorbed at the conclusion of the period of progressive growth, after which the animal developed immunity to implantation of the tumor. We were unable to select the dose of irradiation necessary for obtaining this immunizing effect. After irradiation, the transplantates of M-1 rat sarcoma either would not take on implantation and the animals did not develop immunity, or they took on implantation which grew progressively, leading to death of the animals.

We attempted to create immunity by absorption of an implanted and growing tumor. For this purpose we injected Gordeev's fluid into the substance and beneath the base of an M-1 sarcoma. Despite the fact that in several cases we used doses of the fluid bordering on those lethal for experimental animals (0.15-0.2 ml), the tumors continued to grow progressively and the animals died.

In the next series of experiments necrosis of the tumor was caused by the application of a ligature to its base. F. M. Brikker's recommendation [1] — to tie the ligature not very tightly, in order to embarrass the blood supply of the tumor but not to stop it completely — was not applicable to the tumors investigated. When the M-1 sarcoma was not ligated completely at its base, it rapidly spread under the ligature and continued to grow progressively. We obtained the best results, as in the experiments of Foley [3] and of Keilova and Sorm [5], by ligating the base of the tumor as tightly as possible. The tumor became necrotic and sloughed, whereupon another tumor was implanted into the animal, when it developed, the tumor was again ligated, and this operation was repeated until the animal became completely insusceptible to further implantation of this strain of tumor. Usually the operation had to be repeated 4 or 5 times.

The immune rats were killed by exsanguination and serum was prepared from their blood. At the same time we prepared control sera from the blood of healthy rats which had never received implantation of tumors.

The sera were mixed with equal volumes of a suspension of tumor cells, containing in different experiments from 400,000 to 1 million cells in 0.1 ml of physiological saline. The cell suspensions were prepared as follows: tumor tissue was cut up finely with scissors, physiological saline was added and the suspension was filtered through sterile silk material to remove large aggregates of cells. The cells were counted in a counting chamber and the required concentration of cells was obtained by the addition of physiological saline. The mixtures of cells and sera were injected into rats, after a definite period of contact, in doses of 0.1 ml intradermally

TABLE 1

The Action of an Immune Homologous Serum on Cells of an M-1 Rat Sarcoma

Material implanted	Number of implantations			Mean weight of tumors, in mg
	total	successful	unsuccessful	
Cells of M-1 sarcoma + serum of rats immune to this tumor	25	18	7	1,139
Cells of M-1 sarcoma + control serum	25	23	2	3,782

(experimental and control suspensions into the same animal at symmetrical points on the body). After 2 weeks the rats were killed, and the tumors developing in the animals were excised and weighed.

EXPERIMENTAL RESULTS

It was found that for the sera to display a cytotoxic action, the conditions of their contact with the tumor cells were of great importance. When, before inoculating the rats, we kept the mixture of cells and sera for 30 minutes at 37°, we were unable to find any appreciable difference in the action of the experimental and control sera.

TABLE 2

The Action of an Immune Homologous Serum on Cells of a Sarcoma 465 of Rats

Material implanted	Number of implantations			Mean weight of tumors, in mg
	total	successful	unsuccessful	
Cells of sarcoma 465 + serum of rats immune to this tumor	32	18	14	750
Cells of sarcoma 465 + control serum	32	30	2	2,600

The following experiments were performed differently: the mixtures of cells and sera were kept overnight in the refrigerator and then for 30 minutes at 37°; the rest was done as described above. The results of three similar experiments are summarized in Table 1. It can be seen from the table that the addition of the immune serum to the transplanted cells depressed the chances of a successful take of the tumor and the subsequent growth of the transplantes. Of 25 implantations, 18 experimental ones were successful, by comparison with 23 controls. The mean weight of the experimental tumors was significantly less than that of the controls:

1139 mg compared with 3782 mg. This difference is statistically significant. The probability of chance discrepancy of the mean values (P) is less than 0.001.

In a similar manner we carried out experiments with another rat tumor, sarcoma 465. The results of this experiment were the same (Table 2).

Table 2 is compiled from the combined results of two similar experiments. As may be seen from these findings, the immune sera depressed the chance of successful implantation and growth of sarcoma 465. Of 32 implantations only 18 experimental ones were successful, compared with 30 controls. The mean weight of the experimental tumors was significantly less than that of the controls: 750 mg compared with 2600 mg. This difference was statistically significant, $P < 0.001$.

We were thus able to obtain homologous sera which depressed the growth in vitro of two implanted tumors. In order to judge the role of antibodies in immunity against tumors, however, further experiments are essential, in conditions which exclude the action of isoantibodies.

SUMMARY

The bases of M-1 or 465 sarcomas transplanted to rats were tightly ligated, resulting in necrosis of the tumors. The operation was repeated many times until complete immunity to the inoculation of the above tumors was obtained. The serums of immune rats acquired the property of rendering a definite cytotoxic effect in vitro on the cells of the corresponding tumors, depressing their inoculation ability and growth.

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EXPERIMENTAL BIOLOGY

THE EFFECT OF IONIZING RADIATION OF DIFFERING INTENSITY ON THE REGENERATION OF BONE*

E. M. Pil'shchik

From the Department of Histology and Embryology (Head — Prof. G. S. Strelin) of the Academician I. P. Pavlov First Leningrad Medical Institute

(Received October 21, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The problem of the effect of the chronic, continuous action of radiation on the body has recently acquired great importance.

In spite of the large volume of work [1-7] devoted to the study of the action of ionizing radiation on bone tissue, the question of the chronic action of small doses of radiation on the reparative processes in bone tissue has not yet been adequately dealt with in the literature.

The immediate purpose of our research was to compare the action of the radiation from radioactive cobalt (Co^{60}) when applied once in high intensity and when given as prolonged continuous irradiation of low intensity.

EXPERIMENTAL METHOD

Experiments were carried out on male white rats weighing 150-170 g and ages 5-6 months.

In order to induce a process of regeneration which could be kept under observation, trepanning of the middle third of the diaphysis of the femur was performed on all the animals under aseptic conditions by means of a No. 3 dental drill. By the use of this method it was possible to produce bone defects which were equal in all respects with each other, which was essential for comparison of the material.

The process of healing of the bone wounds was studied in histological preparations taken 3, 5, 7, 9, 12, 14, 18, 21 and 30 days after the operation.

Altogether 3 series of experiments were carried out on 200 animals. In the first series unirradiated animals were used. In the 2nd series a single total exposure to γ-ray irradiation was applied, the doses being 1000 and 1500 r, with a dose rate of 15.5 r/min and exposure times of 3 and 4.5 hours respectively.

Operation was performed on some of these animals immediately after irradiation, and on the others in this series during a period of 3 months after irradiation. These times were chosen for the operation so that the duration of the sequelae of irradiation on bone tissue could be determined.

The animals of the 3rd series were exposed to prolonged, chronic irradiation with γ-rays, which was given in a special room from a special constant-action gun loaded with Co^{60}. The total doses in these experiments (1000 and 1500 r) were of the same magnitude as those given in irradiation of short exposure. The animals irradiated for 12 days (dose rate 125 r/day), for 2 months (25 r/day), 3 months (16.7-11.1 r/day) and 6 months (8.3-5.5 r/day).

*Given at the All-Union Conference on Regeneration and Cell Proliferation on January 28-31, 1958, Moscow.

In comparing the experimental material consideration was taken of the periosteal reaction, the size of the callus formed, and the rate of occurrence of the individual phases of its development. Changes in the bone marrow were also considered.

EXPERIMENTAL RESULTS

The process of healing of the bone wound in the control animals corresponded to the descriptions given in the literature [1, 2, 3], differing only in the times of occurrence of the individual phases of development in accordance with the method of our choice. In the first series, therefore (control animals), we confined our attention mainly to the more precise definition of the times of healing of bone defects.

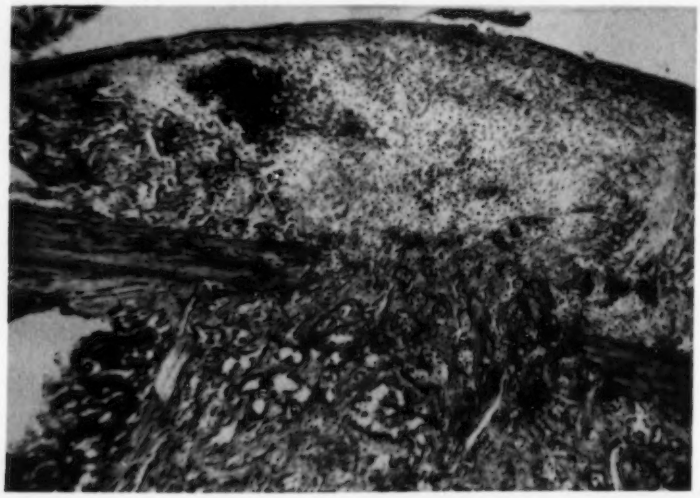

Fig. 1. Regeneration of a bone defect in a control (unirradiated) animal. Time of regeneration 9 days. Microphotograph. Magnification 40 times.

The process of formation of provisional callus in the control animals began on the 3rd-5th day after operation and reached its highest development on the 7th-9th day. At this period differentiation of cells of osteoblastic tissue took place, with the formation of cartilage and of bony trabeculae. Next the reorganization of the provisional callus began, including destruction of cartilaginous tissue and the formation of new elements of woven bone.

This reorganization took place on the 14-15th day of regeneration, after which regression of the callus supervened, with the formation of a marrow cavity, hitherto obliterated.

The microphotograph (Fig. 1) shows the picture of formation of bony callus in a control animal on the 9th day of regeneration. The periosteal callus, covering the site of the defect and consisting mainly of cartilaginous tissue, has reached at this stage the peak of its development. From osteogenic tissue, ingrowing from the side of the periosteum and filling the trepan hole, osteoid and young bony trabeculae have been formed.

An analogous process was also observed in connection with the endosteum, where it was more pronounced and led to obliteration of the marrow cavity. In all parts of the bony callus, parallel with the process of formation of bone tissue, its partial destruction by osteoclasts also took place.

The experiments in which a single total γ-ray irradiation was given showed that, in the doses used (1000 and 1500 r), obvious suppression of the process of regeneration of bone was caused, which was particularly marked when compared with the control animals on the 7th, 9th and 14th days after operation. At later times (21st to 30th days) the differences in the rate of regeneration of the irradiated and unirradiated animals became less pronounced.

The process of formation of bony callus in the irradiated animals passed through the same stages of development as in the controls. It was distinguished, however, by a much slower course. The periosteal part of the callus was constantly of smaller dimensions. In certain cases development of bone tissue took place without

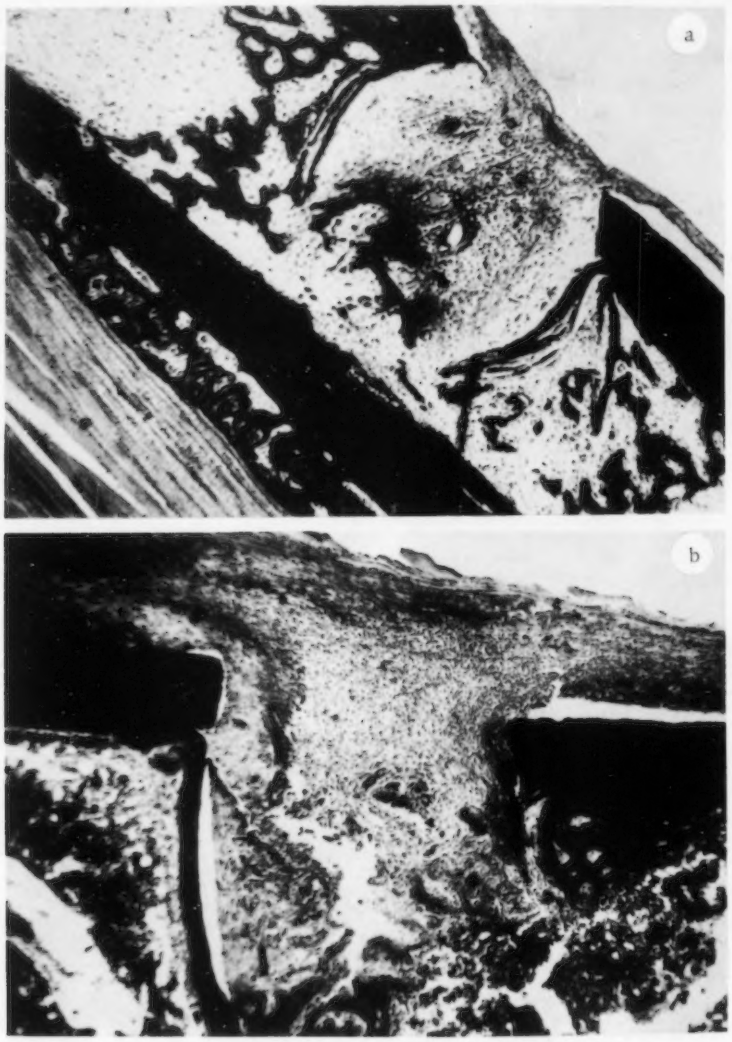

Fig. 2. Regeneration of bone after total γ-ray irradiation with short exposure. a) Operation performed immediately after irradiation; b) operation performed 3 months after irradiation. Dose 1000 r. Time of regeneration 9 days. Microphotograph. Magnification 40 times.

passing through the cartilage. Endosteal osteogenesis was also suppressed. The structure of the bone marrow was grossly disturbed, particularly in the intital period after irradiation.

On the microphotograph (Fig. 2, a) is shown the picture of healing of the bony defect after total γ-ray irradiation of the rat with a short exposure and a dose of 1000 r (the operation was performed 2 hours after irradiation and the time of regeneration was the 9th day). The periosteal part of the callus, covering the bony defect, was almost completely absent. No cartilage tissue was formed in it. The bone wound was filled with undifferentiated granulation tissue. Fibrous bands were predominant in it and recent hemorrhages were often

seen. From time to time islets of chondroid tissue were observed, and young bony trabeculae were seen, but they were smaller in size and fewer in number than in the control animals. No closure of the lumen of the medullary canal took place here. In the preparation, a process of severe impoverishment of the bone marrow in myeloid cells was seen. The remains of the bone marrow tissue consisted mainly of a reticular framework.

Supression of the process of osteogenesis was no less pronounced also in those cases of irradiation with short exposure in which the injury to the bone was inflicted not at the climax of the radiation sickness but 3 months after irradiation (1000 r), i.e., at a period when the signs of radiation sickness, to judge by the peripheral blood picture and other indices, were less marked.

The microphotograph (Fig. 2, b) shows such a picture. It differs little from that shown in Fig. 2, a.

No cartilaginous callus has been formed from the periosteum, the process of differentiation of the cells of the callus into cartilaginous and bony tissue has been severely retarded, and endosteal osteogenesis has been suppressed.

Despite the fact that regeneration of bone tissue was found to be suppressed 3 months after irradiation of short exposure, no severe changes were found in the bone marrow tissue, probably on account of the partial restoration of hemopoiesis.

In the experiments with chronic (continuous) irradiation, lasting 12 days, 2 and 3 months, with total doses of 1000 and 15000 r, the effectiveness of the action of the radiation on the process of regeneration of bone tissue was not lowered, and sometimes was even higher. Prolonged chronic irradiation significantly retarded the process of osteogenesis, which was shown in both earlier (7th-9th day) and later (21st-30th day) stages.

It must be mentioned that the reaction in the bone marrow was feebly expressed. In all the cases of chronic irradiation, the radiation sickness followed a less acute course than after irradiation with the same doses but a short exposure.

On the microphotograph (Fig. 3) is shown the picture of healing of a bone defect after continuous γ-ray irradiation for a period of 2 months (dose 1000 r, time of regeneration 9th day). As may be seen from the microphotograph, the periosteal reaction and the process of endosteal osteogenesis were very feebly expressed,

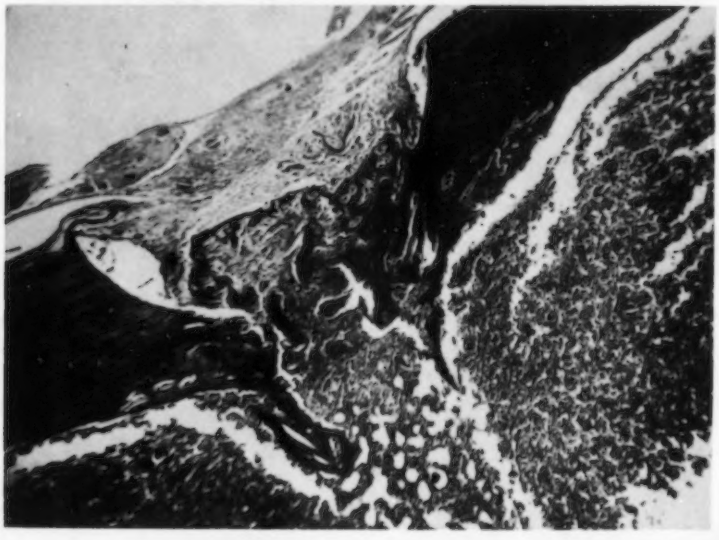

Fig. 3. Regeneration of bone after chronic irradiation for 2 months. Dose 1000 r. Time of regeneration 9th day. Microphotograph. Magnification 40 times.

and the osteogenic trabeculae were few in number and no cartilage tissue was formed. The marrow tissue was compactly filled with cells, among which are seen many megakaryocytes. It has undergone relatively little change.

In the experiments with chronic irradiation lasting 6 months (dose 1000 r), from preliminary findings the suppression of the regenerative power of the bone tissue was more weakly expressed than after less prolonged (for 2 or 3 months) irradiation with the same dose.

Our observations showed that after chronic irradiation with a low intensity, the action of the radiation on the bone accumulated and suppressed the regenerative power of the bone tissue to the same degree as after irradiation with high intensity and short exposure. This occurred even in those cases when the dosage power was considerably reduced and the duration of the irradiation was increased to 3 months.

At the same time it was discovered that the effect of the sequelae of irradiation of short exposure (shown by suppression of the regenerative process) on the bone was very prolonged. From our findings this effect lasted over 3 months. This agrees with the reports in the literature [5, 6, 7].

In contrast to the foregoing, the study of the changes in the bone marrow showed that this suffers only slight damage from chronic irradiation but severe from the single irradiation, although in the latter case its structure was soon restored.

The difference observed in the reaction of the bone tissue and the bone marrow may be explained by their unequal power of repair of the radiation lesion. According to G. S. Strelin [3], such differences are due to peculiarities of the physiological regeneration of the tissues.

SUMMARY

Single radiocobalt (Co^{60}) γ-irradiation of white rats in doses of 1000 and 1500 r caused a distinct suppression of regeneration following trepanation of the femur (the medial third of its diaphysis) by means of a dental drill No. 3 (on the 7th-14th day after the operation). Retarded bone formation could be also observed with the injury of the bone inflicted 3 months after the irradiation (1000 r).

In experiments with chronic irradiation lasting 12 days, 2 and 3 months (the sum total doses being 1000 and 1500 r) the efficacy of the radiation action on the bone tissue would not diminish and at times appeared to be even somewhat higher.

In chronic irradiation the bone marrow tissue showed but slight injuries, but would become acutely disturbed following single irradiation with its structure, however, being rapidly restored in the latter case.

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MITOTIC ACTIVITY IN WHITE RATS IN A STATE OF DRUG-INDUCED SLEEP

L. V. Sokolova

From the Laboratory of Histophysiology (Head — Candidate Biol. Sci. V. N. Dobrokhotov) of the Institute of Experimental Biology (Director — Prof. I. N. Maiskii) of the AMN SSSR, Moscow

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It has been shown that excitation of the body by several different stimuli leads to a lowering of the mitotic activity of certain tissues [4].

The problem of the influence of inhibitory processes in the central nervous system on cell division has not been adequately studied.

Some authors [5, 7] have observed that the tissues of animals which were in a resting state before death were characterized by a higher level of mitosis than those of animals which were in a state of excitation before death. We are aware of the work of Bullough [6] on the effect of barbiturates on the mitotic activity of the epidermis of the ear in white mice. In his opinion, animals in a state of drug-induced sleep show a higher degree of mitotic activity. It must be pointed out, however, that there were several technical defects in this research which might arouse doubts about the results obtained: the counting of the mitotic activity was carried out on several successive pieces of tissue taken from the same animal.

In the present research we endeavored to explain the influence of drug-induced sleep of long and short duration on cell division in several germinating areas of the body.

EXPERIMENTAL METHOD

Investigations were carried out on male rats weighing 100-150 g. The experimental animals were injected subcutaneously with a 2% solution of sodium amytal, in a dose of 0.5 ml/100 g body weight. The control rats were injected with the same volume of physiological saline.

In order to define the state of sleep which usually developed after 5-10 minutes, the corneal reflex of the experimental rats was studied, and the position of the body was noted. The animals were killed by decapitation at the moment of waking; the control rats were killed at the same times.

For histological investigation the tissues were fixed in Zenker's solution, embedded in paraffin wax and stained with hematoxylin by Carazzi's method. The counting of the mitoses was done in every case under the same magnification (objective 90×, ocular 10×). On the cover slips of total preparations of the cornea two perpendicular lines were drawn in ink, crossing at the center of the cornea. The cells and mitoses were counted along these lines. In each case an average of 95 fields of vision were examined. An area of small intestine (10 cm from the outlet from the stomach) was embedded in paraffin wax, and sections were cut from it to a thickness of 8μ. The mitoses were counted in each case in 50 crypts, followed by counting of the cells.

The mitoses in the epidermis of the ear of the rats were counted in serial sections 8μ in thickness (alternate sections). In each case 6000-8000 cells were examined altogether. The mitotic coefficient per 1000 was calculated in relation to the total number of cells. By comparison of the mitotic coefficients the probability (P) of random difference in their values was determined by the Fisher-Student method [3].

EXPERIMENTAL RESULTS

As may be seen from Table 1, after a single injection of a hypnotic drug during the morning and afternoon (10 A.M. to 2 P.M.) there was no change in the mitotic coefficient in the cornea, skin and intestine.

Under these circumstances, however, considerable individual variations in the value of the mitotic coefficient were observed. These variations were evidently due to the fact that animals in which the sleep had lasted for different lengths of time were killed at the same time of day (from 11 A.M. to 6 P.M.). It is known that the mitotic activity in rats in the morning and evening hours is different [2].

We accordingly carried out an additional experiment in which a single injection of the hypnotic drug was given at a time when the mitotic activity was low (5 P.M.). The animals were killed at 8-9 P.M. In this experiment the mitotic coefficient was determined in the corneal epithelium alone. The results of the experiment (Table 2) showed that in the evening hours after a single injection of the hypnotic drug causing induced sleep, the mitotic coefficient in the experimental animals was slightly increased by comparison with that of the controls. This increase was, however, small and was not statistically significant.

In order to ascertain what changes took place in the mitotic activity after prolonged, continuous drug-induced sleep, we carried out a second series of experiments. Sleep lasting 10-11 hours was produced by repeated injections of sodium amytal. Each successive injection was given at the moment when the animal was emerging from sleep. The control animals were given a single injection of physiological saline in order to avoid the possibility of a lowering of the mitotic activity in response to the needle. According to the number of injections the animals were divided into 4 groups.

TABLE 1

Mitotic Coefficient (per 1000) in the Epithelium of the Cornea, the Epidermis of the Ear and the Crypts of the Small Intestine of White Rats after a Single Injection of Sodium Amytal during the Morning Hours

Duration of sleep (in hr)	Group of rats	Number of animals	Mitotic coefficient		
			cornea	skin	intestine
1	Experiment	6	11.6	3.5	28.4
	Control	6	10.1	3.1	27.8
2	Experiment	10	7.5	1.8	25.5
	Control	6	8.5	3.6	27.9
3	Experiment	8	9.7	1.6	24.1
	Control	8	7.4	2.1	26.9
4	Experiment	6	6.2	2.4	26.9
	Control	4	5.8	2.5	30.9

The results of this experiment, given in Table 3, show that after drug-induced sleep lasting continuously for 10-11 hours, a significant increase was observed in the number of cell divisions in the corneal epithelium of the experimental animals by comparison with the controls (in all the groups P varied between 0.04 and 0.001). This stimulation of mitotic activity did not depend on the number of injections of sodium amytal given in the course of the experiment.

From the counting of the mitoses in the epithelium of the ear no such difference was revealed, although the experimental animals showed a certain tendency to increase of the mitotic coefficient by comparison with

the controls. No changes were observed in the mitotic activity of the epithelium of the crypts of the small intestine after prolonged, continuous drug-induced sleep.

Irrespective of the duration of the sleep, the differing depth of the sleep in the experimental animals was evidently accounted for by their typological peculiarities. On these grounds it was possible to subdivide all the experimental animals of the second series into two groups: the first included rats in which a state of deep sleep was observed, and the second rats with only superficial sleep. It must be pointed out that the sleep of longest duration occurred in the animals after the second injection of the hypnotic drug. After subsequent injections the duration of sleep decreased, presumably because habituation developed. The results of this experiment are shown in Table 4.

TABLE 2

Mitotic Coefficient (per 1000) in the Corneal Epithelium of White Rats after a Single Injection of Sodium Amytal during Evening Hours

Duration of sleep	Experiment		Control	
	number of animals	mitotic coefficient	number of animals	mitotic coefficient
1 hour 35 minutes	4	8.80	—	—
2 hours 30 minutes	17	4.91	9	4.23
3-4 hours	10	6.15	10	5.72
Mean	—	6.6	—	5.0

As may be seen from Table 4, the mitotic activity of the corneal epithelium of the group of animals in deep sleep was almost twice as high as in the corneal epithelium of the control rats ($P < 0.0001$). In the group of animals with superficial sleep the mitotic activity in the cornea was also higher than in the control animals, but this increase was not significant ($P = 0.57$). On the other hand, by comparison of the mitotic coefficients of the corneal epithelium of the rats in these two groups, a statistically significant difference was found in the number of mitoses ($P = 0.0007$). The mitotic coefficient of the epidermis of the ear was higher in the animals with deep sleep than in those with superficial, and it differed from that in the control animals but this

TABLE 3

Mitotic Coefficient (per 1000) in the Corneal Epithelium, the Epidermis of the Ear and the Epithelium of the Crypts of the Small Intestine of White Rats after the Repeated Injection of Sodium Amytal during Evening Hours

No. of injections	Cornea				Skin				Intestine			
	experiment		control		experiment		control		experiment		control	
	number of animals	mitotic coefficient	number of animals	mitotic coefficient	number of animals	mitotic coefficient	number of animals	mitotic coefficient	number of animals	mitotic coefficient	number of animals	mitotic coefficient
I			24	5.3			23	2.1			22	25.7
II	4	8.9			4	2.5			3	24.9		
III	13	7.7			13	2.4			13	28.8		
IV	16	8.6			16	2.9			16	21.6		
V	7	8.6			7	2.5			7	26.8		

increase was not statistically significant (P = 0.19). No changes in the mitotic activity in relation to the depth of sleep were observed in the epithelium of the crypts of the small intestine.

Counting of the mitoses by phases showed predominance of the earlier phases of mitosis in the experimental animals of this series by comparison with the controls. The ratio between the numbers of early and late phases in the cornea of the control animals, for instance, was 1.0, and in the experimental animals 1.3-1.5, irrespective of the number of injections of hypnotic drug and the depth of sleep.

The findings described thus show that sleep of short duration after a single injection of hypnotic drug, whether in the morning (10 A.M. to 1 P.M.) or evening (5 P.M.) hours, had no effect on the level of mitotic activity in the corneal epithelium of white rats.

Prolonged, continuous drug-induced sleep, after repeated injections of sodium amytal, caused an increase in the mitotic coefficient in the epidermis of the ear and had no effect on the mitotic activity of the epithelium of the crypts of the small intestine. The increase in the number of cell divisions in the corneal epithelium depended on the depth of the animal's sleep; in the epidermis of the ear this relationship was expressed to a lesser degree, and in the intestine it was not found.

The greater increase in mitotic activity in the corneal epithelium than in the other germinating areas (skin, intestine) was evidently due to its greater reactivity to the most diverse agents. It is also known that the cornea responds by a considerable lowering of its mitotic activity to the injection of adrenalin, whereas the intestine

TABLE 4

Mitotic Coefficient (per 1000) in the Corneal Epithelium, the Epidermis of the Ear and the Epithelium of the Crypts of the Small Intestine of White Rats after Emerging from a State of Deep and Superficial Drug-Induced Sleep

Test object	Control		Sleep			
			superficial		deep	
	number of animals	mitotic coefficient	number of animals	mitotic coefficient	number of animals	mitotic coefficient
Cornea	24	5.3	20	7.0	20	9.7
Epidermis	23	2.1	20	2.3	20	2.9
Intestine	22	25.7	20	28.2	19	26.1

does not react to this drug [1, 4]. The epidermis showed the same tendency to increase of mitotic activity as did the corneal epithelium, but the corresponding changes were expressed to a lesser degree. It is not ruled out that the dose of hypnotic causing changes in the cornea was inadequate for the epidermis.

It may be postulated from the results obtained that changes in the intensity of cell division are dependent on the fundamental processes taking place in the central nervous system.

SUMMARY

Single administration of hypnotics in the morning or in the evening induces no changes in the mitotic activity of the cornea, skin and crypts of the small intestine. Prolonged continuous drug-induced sleep for a period of 10-11 hours causes a rise of the mitotic activity in the cornea, slightly increases the mitotic activity in the ear epidermis, but leaves it unchanged in the crypts of the small intestine. The rise of mitotic activity in the corneal epithelium depends on the profoundness of the animal's sleep.

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THE EFFECT OF DRUG-INDUCED SLEEP ON THE MITOTIC ACTIVITY OF THE CORNEAL EPITHELIUM OF WHITE MICE

S. Ya. Zalkind

From the Moscow Research Institute for Antipoliomyelitis Preparations

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It may be considered firmly established that the state of the nervous system has an essential influence on the mitotic regime of the body. We have shown that excitation of the nervous system, caused by a moderately painful stimulus, leads to a temporary fall in the mitotic activity of the corneal epithelium [1].

The question naturally arose of the influence on the mitotic regime of the opposite state — lowering of the excitation of the nervous system caused by the use of substances leading the animal into a state of artificial, or drug-induced sleep.

The performance of investigations along such lines is all the more justified by the fact that there are indications in the literature [2] that drug-induced sleep has a favorable effect on the course of tissue regeneration. It has been found that the use of small doses and microdoses of a number of narcotics has a stimulating action on the healing of skin wounds in rats, and that under these circumstances the proliferative process begin sooner than in control animals.

We investigated the effect of artificial sleep of short duration, induced by the subcutaneous injection of sodium amytal, on the mitotic activity of the corneal epithelium.

EXPERIMENTAL METHOD

White mice (weighing 14-16 g) were given subcutaneous injections of 0.6 ml of a 0.4% solution of sodium amytal. After 15 minutes they fell asleep. While they were asleep, 3 hours after the injection, the animals were killed by rapid decapitation, and the cornea, with the rest of the eye, was fixed in a mixture of 70° alcohol with 5% acetic acid. Animals of the control group, which received injections of 0.6 ml of physiological saline, were killed at the same time. The subsequent treatment, preparation of total specimens of the cornea and the method of counting mitoses were described in our previous paper [1]. The mitoses were counted in the whole area of the cornea, but the mitotic coefficient was derived from consideration of 100 fields of vision. At the same time as the mitoses were counted the phase coefficient — the ratio between the number of prophases + metaphases to the number of anaphases + telophases — was determined.

EXPERIMENTAL RESULTS

The results obtained are shown in summarized form in the table, which was compiled on the basis of 7 animals in each group (100 fields of vision in each case).

As may be seen from the figures in the table, drug-induced sleep of three hours' duration caused a sharp increase in the number of mitoses in the mice (by 104%). The phase coefficient, which for the control animals was equal to 1.2, rose in the experimental animals to 1.88, which indicated the considerable predominance of early phases of mitosis (prophase and metaphase), i.e., an increase in the number of cells embarking on the process of cell division.

These findings suggest that drug-induced sleep caused stimulation of mitotic activity. In these circumstances sleep had an effect which was directly opposite to that observed in response to excitation of the nervous system caused by painful stimulation [1].

Changes in the Mitotic Activity of the Corneal Epithelium of White Mice under the Influence of Drug-Induced Sleep

Control				Experiment			
prophases 79.8	metaphases 147	anaphases 88.8	telophases 99.4	prophases 205	metaphases 227	anaphases 177.6	telophases 126.7
Sum – 415				Sum – 847			
Phase coefficient $\frac{P+M}{A+T} = 1.2$				Phase coefficient $\frac{P+M}{A+T} = 1.88$			

It is still too early to give an exhaustive explanation of our findings. There are indications in the literature that the use of drug-induced sleep also leads to considerable changes in the metabolism of the body.

It has been shown by the use of labeled methionine that, during drug-induced sleep, an increase takes place in the intensity of incorporation of amino acids in the serum proteins and in a number of organs – the liver, adrenals and cardiac muscle [3].

Controlled experiments revealed that the narcotic drugs themselves (sodium amytal, urethane with veronal) in the doses used did not cause metabolic changes, which were thus associated with the narcotic state itself [4].

From our point of view it was of particular importance that during drug-induced sleep in rabbits a fall was observed in the blood sugar and in increase in the glycogen content of the muscles and liver [5]. The results of this investigation were interesting because, according to Bullough's findings [6, 7], these changes in carbohydrate metabolism were characteristic of an increase in the mitotic activity of the epidermis of the ear in mice.

It is possible, however, that there is a somewhat different explanation of our findings. It may be postulated that the weakening of the influence of the nervous system, as found in the state of artificial sleep, directly or indirectly (possibly by means of a change in the adrenal regime of the body) leads to weakening of the normally present depressing influence of the nervous system on mitotic activity.

Thus although the mechanism of the stimulation which we observed in the mitotic activity of the corneal epithelium cannot yet be regarded as finally explained, the results of physiological and biochemical investigations of the influence of drug-induced sleep on the body are in substantial agreement with our findings.*

SUMMARY

The author studied the effect of drug-induced sleep of short duration on the mitotic activity corneal epithelium (0.6 ml of 0.4% sodium amytal solution). The number of mitoses rose by 10% after 3 hours' sleep. The phasic coefficient rose in the experimental rats up to 1.88%.

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CHANGES IN THE ACCESSORY SEX GLANDS OF THE MALE RABBIT DUE TO SYNESTROL

A. G. Bobkov

From the Department of Pathological Anatomy (Head – Prof. M. A. Zakhar'evskaya) of the I. P. Pavlov First Leningrad Medical Institute (Director – Docent A. I. Ivanov)

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A considerable volume of research has been published in the literature, devoted to the effect of the estrogens on the male accessory sex glands [4-9, 11, 13 and others]. In spite of reports of dissimilar changes in these glands in response to the injection of estrogens to animals of various species [6, 7], no experiments have been carried out on rabbits. Furthermore, having observed inflammatory changes in the accessory sex glands from the action of hormones, the majority of authors have not devoted sufficient attention to them, nor have they considered the question of the possible effect of inflammation on the changes in the glands caused by administration of the estrogen. É. Z. Yusfina alone [10] points out the combined stimulating action of the estrogen (folliculin) and of inflammation on the growth of the tissue cells of the prostate gland of a rabbit, implanted by F. M. Lazarenko's method. Another estrogen – synestrol (hexestrol) – in É. Z. Yusfina's experiments inhibited the inflammatory growth of the epithelium, but this contradiction must have been due to the very large dose of synestrol which led to rapid proliferation of the connective tissue.

Having obtained some information on the inflammatory changes in the epithelium of the accessory sex glands of the male rabbit [1], we nominated the next step in the research to be comparison of these with the hormonal changes.

EXPERIMENTAL METHOD

Experiments were carried out on 22 young male rabbits, aged about 5 months and weighing from 1400 to 2200 g. Nine of these rabbits were subjected to preliminary castration. Every other day the rabbits were regularly injected with synestrol subcutaneously in 2% oily solution. For a period of 81 days the dose injected was 0.3 ml (0.006 g of pure synestrol), after which the sessional dose was increased to 0.45 ml (0.009 g). The times of the experiments were as follows: 4, 8, 12, 16, 22, 28, 36, 37, 60, 75, 97, 109, 122, 129, 143, 156, 159, 173, 179, 186, 198 and 211 days. The material was fixed in formalin and embedded in celloidin and paraffin wax. Histological sections were stained by Van Gieson's method and with hematoxylin-eosin, and some sections were also stained by Gram's method and mucicarmine.

EXPERIMENTAL RESULTS

The administration of considerable doses of synestrol caused wasting of the rabbits. The external appearance of the accessory sex glands was only slightly altered. Usually at first the glands were slightly reduced in size, and in the later periods of the experiment (4-5 months) they increased in size.

In early periods of the experiment (4-8 days) an unevenly marked hypermia and edema of the stroma developed, which increased in intensity until the 8th day, and then gradually diminished. On the 8th day, leucocytes accumulated in the vessels, and then migrated into the surrounding tissue. Meanwhile an increase took place in the numbers of histiocytes and lymphoid cells in the connective tissue. Infiltrative phenomena were found

primarily on the mucous membrane of the urethra and in the prostate gland, and then in the vesiculiform* and other glands. The leucocytes penetrated into the epithelium and accumulated in the lumen of the cavities of the glands. In the urethra, the leucocytic infiltration of the mucosa was observed at the level of the vesiculiform gland and more distally; in the more proximal segments it was absent. In the epithelium of the glands secretion was diminished and came to an end. Swelling of the cells of the basal row took place, so that they became enlarged and rounded. In some cells a vacuolar dystrophy was observed. At the same time, even at these stages a weak proliferation of the epithelium could be detected, as a result of which, on the 9th day of the experiment, the epithelial cells formed, in places, 3 or 4 rows. The proliferation of the epithelium had a focal character and usually was independent of the degree of expression of the infiltrative changes. In the castrated animals proliferation was observed sooner and developed at a more rapid rate, although considerable individual variations were observed in the rate of its development.

As a result of the proliferation a thickening of the epithelial layer took place, with the formation of structures of transitional and stratified squamous epithelium. We first observed structures resembling transitional epithelium on the 16th day of the experiment in a castrated rabbit, in the ducts of the vesiculiform, prostate and upper Cowper's glands. This epithelium consisted of 2 or 3 rows of light, imbricate cells. At the base of the epithelium was differentiated a layer of smaller cells, and on its surface there was a layer of cubical or flattened cells, which were the cells of the superficial layer of the pre-existing two-layered epithelium. Stratified squamous epithelium with keratinization and desquamation of keratinized layers was first observed on the 37th day of the experiment in solitary acini of the vesiculiform gland, together with insignificant signs of proliferation

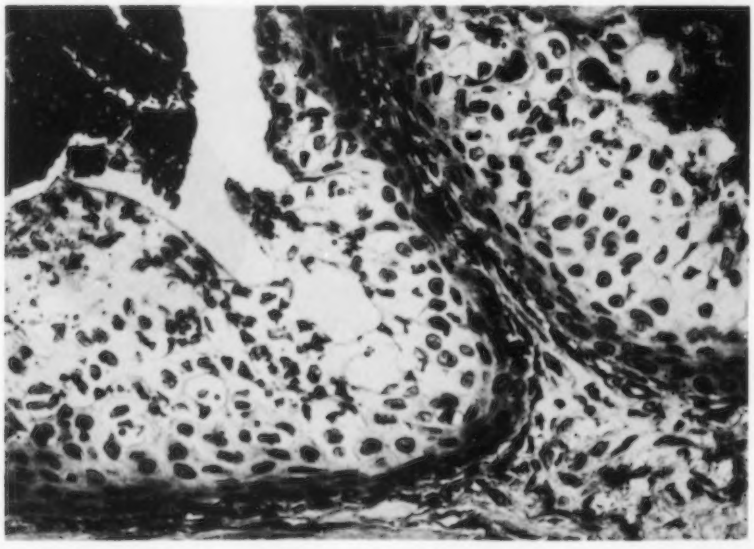

Fig. 1. Experiment at 122 days (0.426 g synestrol). Differentiation of stratified squamous epithelium at the base of a layer of degenerating transitional epithelium in the vesiculiform gland. Van Gieson's stain.

of the epithelium in other cavities. The formation of transitional and stratified squamous epithelium became a constant sign only later – 2 months and more from the onset of administration of synestrol.

In the vesiculiform and prostate glands transitional epithelium was formed in the majority of the acini of the glands. Areas of thickened epithelium which had not attained the degree of differentiation of the transitional, and areas of two-layered epithelium could, however, always be found. In places, at the base of the layers of transitional epithelium, differentiation of stratified squamous epithelium was observed. At such places the upper zone of the epithelial layer consisted of large, light imbricate cells, corresponding to transitional epithelium, and the lower was composed of compact, polygonal or flattened cells with a sharply outlined basal layer of small cubical cells (Fig. 1). In these layers, the transitional epithelium had completely desquamated in places, and was replaced by stratified squamous nonkeratinizing epithelium.

* The glandula vesicularis found at the neck of the bladder. – Publisher.

Proliferation of the epithelium of the upper Cowper's gland began in the major ducts. Gradually the process spread to involve the epithelium of the smaller ducts. The terminal divisions remained intact for a long time, but at 2 months their epithelium had also begun to proliferate. In these ducts, transitional and stratified squamous epithelium developed at the same times of the experiment and in the same combinations with each other as in the vesiculiform and prostate glands. The layers of stratified squamous epithelium in the upper Cowper's gland were highly developed, and in places consisted of 20 or more layers of cells.

In the seminal vesicle the distal divisions in normal conditions are often lined with transitional epithelium too. For this reason the designation of structures composed of transitional epithelium here as new formations resulting from the action of synestrol must be made with care. Nevertheless, under experimental conditions, transitional epithelium was found in the seminal vesicle more often and at a higher level, sometimes throughout its whole extent, which was not observed without the action of synestrol. The frequently found mitoses were further evidence of proliferation of the epithelium of the vesicle. Sometimes two sharply delineated zones could be distinguished in the transitional epithelium of the seminal vesicle: a superficial consisting of large, light cells, and a deep, formed of several rows of far smaller and darker cells. On the basis of comparison with the two-layered structures of transitional and stratified squamous epithelium of the other glands described above, these appearances in the seminal vesicle must evidently be regarded as a tendency toward the formation of stratified squamous epithelium. However, the latter was found very rarely in the seminal vesicle. Only in one

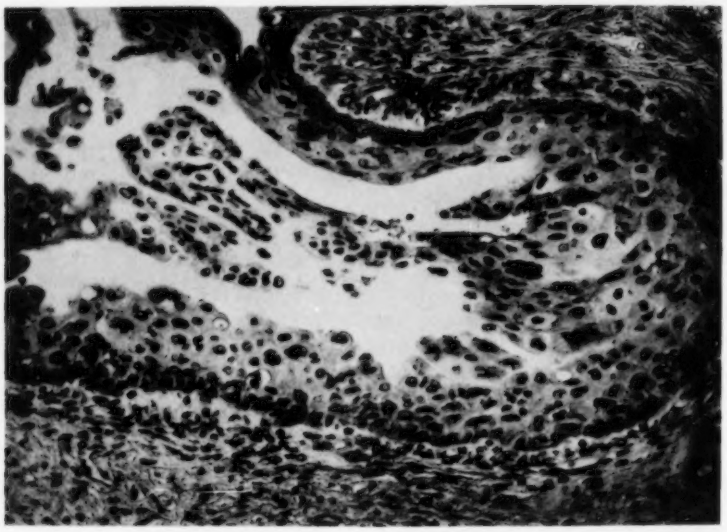

Fig. 2. Experiment at 60 days (0.18 g synestrol). Atypia and polymorphism of the cells in areas of proliferation of the epithelium of the seminal vesicle. Multinuclear epithelial cells. Van Gieson's stain.

case was it possible to observe typical stratified squamous nonkeratinizing epithelium in those areas of the seminal vesicle where subacute productive inflammation was well marked underneath the epithelium. Adjoining these areas destruction of the epithelial lining of the vesicle by the inflammatory process was observed.

Sometimes the proliferation of the epithelium of the seminal vesicle led to the formation of solid epithelial outgrowths, projecting into the lumen. These outgrowths were characterized by gross atypia and polymorphism, and consisted of cells and nuclei of a variety of shapes and sizes, of nuclei with different staining properties and of multinuclear epithelial cells. These tissues were reminiscent of carcinoma, but no infiltrative growth was observed (Fig. 2).

In the lower Cowper's gland transitional, and sometimes stratified squamous nonkeratinizing epithelium, formed the normal lining of the distal divisions of the ducts. In the experiments the layers were observed to be

much thicker, and the structures mentioned were formed in the medium-sized ducts. No proliferation of the epithelium of the small ducts and terminal divisions was observed.

In the ampullae of the seminiferous tubules the epithelium preserved its two-layered structure. In only one case, when in the membrane itself obvious inflammation was observed, was the epithelium thickened and permeated with leucocytes, consisting of 3 or 4 rows of lightly stained cells.

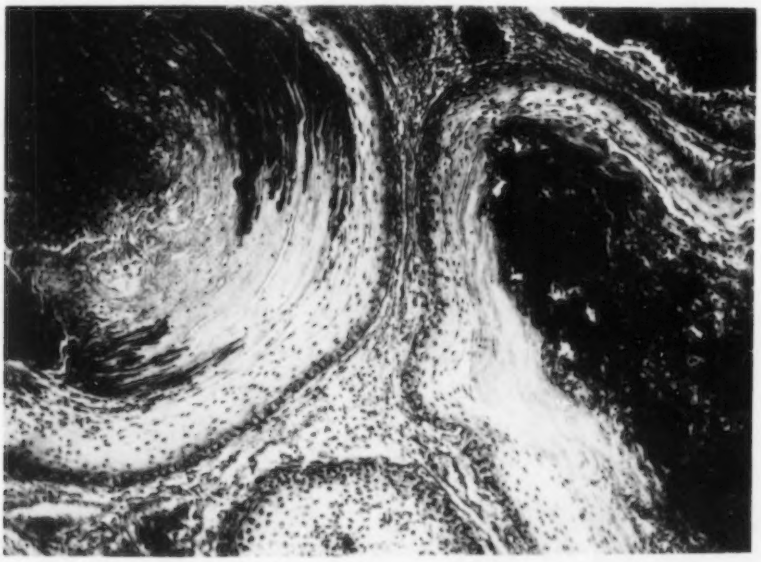

Fig. 3. Experiment at 186 days (0.705 g synestrol). Stratified squamous epithelium with marked keratinization in the vesiculiform gland. Van Gieson's stain.

The stratified squamous epithelium of the vesiculiform, prostate and upper Cowper's glands was affected by keratinization (Fig. 3). The keratin scales desquamated into the lumen. The accumulation in the lumen of desquamated epithelial cells, masses of keratin and disintegrating leucocytes led to gross dilation of the acini of the glands, which sometimes reached a diameter of 0.3-0.5 cm. Dilatation of the cavities caused enlargement of the particular glands.

In late stages of the experiment (5-6 months) sclerosis of the stroma of the accessory sex glands developed, with atrophy of muscle bundles and epithelium. Evidently in the conditions of these experiments acellular sclerosis was predominant, since no significant proliferation of fibroblasts was observed.

Signs of degeneration became more intense in the epithelial layers. Many cells became infiltrated with fat and were converted into pseudoxanthomatous cells with a very lightly stained, scanty protoplasm. The cells of the stratified squamous epithelium which did not undergo keratinization were desquamated in large numbers; their nuclei showed pyknosis and rhexis, but alongside degeneration of some cells mitoses were found in others, demonstrating the continuing growth.

The dissimilar changes in the epithelium of the different glands under the influence of synestrol were evidence of specific features in the morphological and functional differentiation of the epithelium in various parts of the reproductive system.

After administration of synestrol, dystrophic, exudative-infiltrative and proliferative phenomena were combined in the glands, and hence, from its formal signs, the process as a whole could be described as inflammation [12]. Proliferation of epithelium could not, however, be equated with ordinary inflammatory growth as distinguished by V. G. Garshin [3], since no correspondence was usually observed between the proliferation of the epithelium and the degree of expression of the inflammatory changes in the underlying tissue. Furthermore, under the influence of synestrol, the formation of stratified squamous epithelium and keratinization of this

epithelium were expressed far more intensively than in inflammation. A hormonal origin must therefore be accepted for the epithelial proliferation studied in this research. In spite of the fact that hormonal growth of epithelium is an independent form of growth, independent of inflammation, the latter may, nevertheless, affect hormonal growth. This must account for the formation of stratified squamous epithelium in the seminal vesicle only in the presence of inflammation, whereas in the absence of inflammation no squamous cell structures developed in this gland.

In the conditions of these experiments no transformation of hormonal epithelial growth into blastomatous was observed. This was in agreement with the findings in the literature [2].

SUMMARY

Epithelial proliferation associated with dystrophic changes, inflammatory infiltration and sclerosis of the stroma (at the late periods of experiment) are observed in the accessory sexual glands of male rabbits as the result of subcutaneous injections of synestrol. Epithelial proliferation is frequently associated with its metaplasia into transitional and stratified squamous types with casual cornification. The growth of the epithelium induced by synestrol injection is independent of the inflammation. However, the latter may influence this growth.

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MORPHOLOGY AND PATHOMORPHOLOGY

THE RELATIONSHIPS BETWEEN NERVE CELLS AND CAPILLARIES IN THE PHRENIC NERVE NUCLEUS AND THE ANTEROLATERAL GROUP OF CELLS IN THE SPINAL CORD OF THE ADULT CAT

N. G. Palenova

From the Division of Development of the Brain (Head – Corresponding Member AMN SSSR Prof. B. N. Klosovskii) of the Institute of Pediatrics (Director – Corresponding Member AMN SSSR Prof. O. D. Sokolova-Ponomareva) of the AMN SSSR, Moscow

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The systematic study of the blood supply of the various divisions of the brain and investigation of the relationship between the nerve cells and capillaries in these divisions both in adult animals and in the course of individual development [1-7] have led to the necessity of elucidating the special features of vascularization of the spinal cord by comparison with other divisions of the central nervous system from a consideration of individual nuclei and conducting pathways.

In this paper we present the results of research into the relationships between the nerve cells and capillaries in the anterior horns of the cervical division of the spinal cord of the adult cat.

The investigation was carried out on motor nuclei of the spinal cord with different functions, namely on the nucleus of the phrenic nerve, giving off its axons to the diaphragm, and on the anterolateral group of cells innervating part of the muscles of the shoulder girdle. The difference in function of these nuclei is brought about by the fact that the phrenic nerve nucleus in adult animals transmits rhythmic impulses to the diaphragm, whereas the anterolateral group of cells works irregularly and is in a state of activity for varying intervals of time.

EXPERIMENTAL METHOD

In order to study the relationships between the nerve cells and capillaries in the phrenic nerve nucleus and the anterolateral group of cells, we used continuous series of laterolateral longitudinal sections of the 5th-6th segments of the cervical division of the spinal cord of adult cats, the vascular network of which was injected with ink and gelatin, and the nerve cells stained with thionin.

The special features of the relationships between nerve cells and capillaries surrounding them were investigated by the routine method adopted in the laboratory of brain development. Under these circumstances the following signs were taken into consideration: the total length of the capillary network surrounding the cell to a distance of 25 μ the arrangement of the capillaries around the cell the number of cells in contact with capillaries, the extent of their contact, the distinctive arrangement of the capillary on the body of the nerve cell and the length of the genu of the capillary.

EXPERIMENTAL RESULTS

Before embarking on the description of the relationships between the nerve cells and capillaries in the motor nuclei of the anterior horns of the spinal cord, we must point out the considerable differences in the structure of the nuclei which we were studying and in the distribution of their capillary network.

Some authors [8, 9] have drawn attention to the fact that the phrenic nerve nucleus of the cat consists of unconnected groups of cells, drawn out in the form of a rod, in a direction parallel to the anterior longitudinal sulcus in the 5th-6th segments of the cervical division of the spinal cord. The cells of this nucleus are fusiform; besides large cells, small numbers of small cells are found in the nucleus, which are considered by these authors to be intercalary neurones. The dendrites of the cells are also directed longitudinally and form a dendritic "tract".

More detailed investigation has shown that the cells in each group lie close to each other, at a distance of less than 25 μ. They have a somatochrome type of structure. They vary in length from 80 to 150 μ and in width from 30 to 60 μ. The small cells forming a part of the nucleus also have a somatochrome type of structure. They measure 20-30 μ in length and 16-20 μ in width.

The characteristic feature of the structure of the cells of the phrenic nerve nucleus was the emergence of their processes in the majority of cases from the upper and lower poles of the cell (Fig. 1, a). As has already been mentioned, the cell processes formed a tract, directed along the long axis of the spinal cord. In contrast

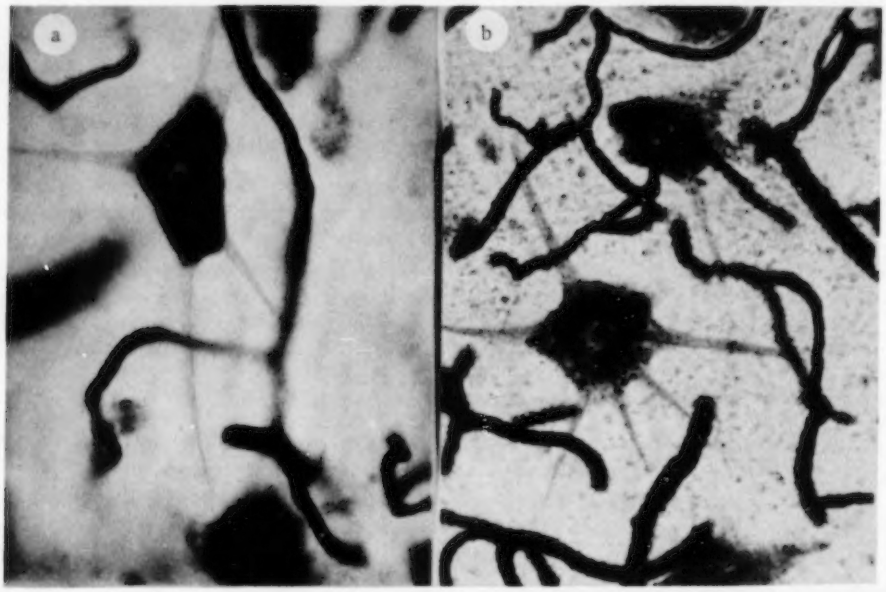

Fig. 1. Relationship of nerve cells to capillaries in the anterior horns of the spinal cord of the adult cat. a) Cell and capillary from the nucleus of the phrenic nerve; b) cell and capillary from the anterolateral group. Vascular network injected with ink and gelatin. Nerve cells stained with thionin. Magnification 400 times.

to the phrenic nerve nucleus, the cells of the anterolateral group were polygonal in shape and not above 60-70 μ in size. The cell processes had no particular orientation and went in various directions (Fig. 1, b). The characteristic feature of the structure of this group was the even distribution of the nerve cells in it, situated at a distance of more than 25 μ from each other.

The cell groups which we studied differed from each other not only by the arrangement of the nerve cells and their processes within them, but also by the individual structure of their vascular network and by the intimate relationships between the nerve cells and capillaries. The capillary network in the nucleus of the phrenic nerve, for instance, consisted of loops drawn out along the long axis of the spinal cord, and moreover the length of the capillary genu forming the loop and situated in the long axis of the brain reached 300 μ, but the capillaries situated in the transverse direction in the spinal cord were only 80-90 μ in length.

The capillaries in the phrenic nerve nucleus of the adult cat were characteristized by even and clear outlines. In preparations whose vascular network was injected with ink, their diameter was 4-5 μ. In these preparations it could be seen that, on account of the longitudinal arrangement of the vascular network, the phrenic nucleus could be distinguished clearly from the neighboring cell group (Fig. 2, a).

On comparison of the arrangement of the nerve cells and capillaries in the phrenic nerve nucleus, it could thus be observed that the longitudinal direction of the capillary network in the nucleus corresponded to the arrangement of the nerve cells therein.

As has already been pointed out, the cells in the phrenic nerve nucleus were arranged in groups. For this reason one capillary, passing to such a group, could simultaneously supply 2 or 3 cells. The capillaries around the cells formed open loops, stretched out along the cell bodies. It must be pointed out that in a few cases the

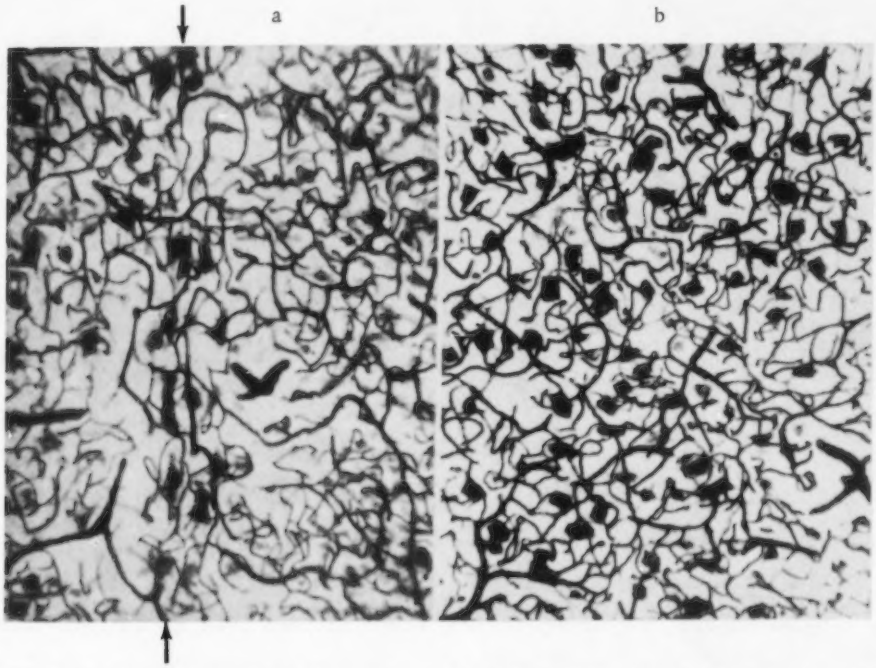

Fig. 2. Angioarchitectonics of the motor nuclei of the anterior horns of the spinal cord of the cat. Laterolateral longitudinal sections. a) Nucleus of the phrenic nerve, situated along the whole length of the preparation; b) anterolateral group of cells. Vascular system injected with ink and gelatin. Nerve cells stained with thionin. Magnification 80 times.

capillaries were in contact with the bodies of the cells (1-2 cells from each 10). The extent of contact of the capillary lying on the surface of the cell body amounted to 15% of the length of its perimeter.

In the phrenic nucleus we observed a special feature in the relationship of the capillaries to the cell processes. In some cases we could observe capillaries which accompanied the cell processes for a long distance. Moreover capillaries were seen to pass parallel to bands formed of a number of cell processes, at a distance of less than 25 μ from them. E. N. Kosmarskaya and E. G. Balashova [7] also observed that processes of nerve cells were accompanied by capillaries in the reticular substance of the medulla oblongata of the cat.

A different structure of the capillary network and relationship of nerve cells to capillaries was observed in the anterolateral group (Fig. 2, b). In contrast to the phrenic nerve nucleus, the capillaries in this group had no definite orientation and went in different directions. The length of the genu of the capillaries was roughly the same in all their planes of distribution, and was on the average 100-110 μ, so that the capillary loops were

polygonal in shape; they were composed of vessels having clear outlines and a uniform lumen. In preparations in which the vascular network was injected with ink, their diameter varied from 4 to 5 μ.

As has already been stated, the cells in the anterolateral group were separated from each other by a distance of over 25 μ. The capillaries arranged around each cell were therefore concerned with that cell alone, whereas in the group of cells of the phrenic nerve nucleus, as we have mentioned, one capillary supplied several cells. In contrast to the phrenic nerve nucleus, of each 10 cells of the anterolateral group, 6 cells were in contact with capillaries, and moreover the capillary was applied to the body of the cell for a comparatively long distance. The extent of contact of a capillary lying on the surface of the cell body amounted to 29% of the length of its perimeter.

On the example of the motor nuclei of the anterior horns of the spinal cord it could thus be seen that nuclei of different function, with sharp differences in their structure, were vascularized in a different manner. The nerve cells composing these nuclei had different relationships with the capillaries surrounding them.

SUMMARY

In an adult cat the phrenic nucleus and the anterolateral group of cells (the functionally different nuclei of the anterior horns of the spinal cord) differ not only by their structure, but also by vascularization of their cellular constituents. The structure of capillary network in the nucleus of the phrenic nerve corresponds to the longitudinal distribution of the nerve cells and their processes in the nucleus. In group distribution of the nerve cells in the nucleus of the phrenic nerve, each capillary simultaneously serves several cells of one group of nerve cells, without coming into contact with their bodies (in the majority of cases). In the event of a contact, the linear contact does not exceed 15% of the cellular perimeter. In contradistinction to the nucleus of a phrenic nerve, the capillary network in the anterolateral group has no definite orientation. The nerve cells in the latter are distributed uniformly and located at a distance of more than 25 micron from each other. Therefore, the capillaries located around each cell pertain to this cell. The number of cells in contact with capillaries and the linear extension of contact is greater in the anterolateral group than in the nucleus of the phrenic nerve.

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METHODS

AN ULTRAMICROMETHOD OF DETERMINING THE TOTAL NITROGEN

I. B. Krasnov

From the Laboratory of Histochemistry (Head – Prof. V. V. Portugalov) of the Institute of the Brain (Director – Active Member AMN SSSR S. A. Sarkisov) of the AMN SSSR, Moscow

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The necessity of biochemical investigation of small quantities of biological material calls for the use of micro- and ultramicromethods, one of which is an ultramicromethod of determination of the total nitrogen.

Methods of determination of the total nitrogen in quantities of the order of micrograms which have so far been described [3, 4, 5, 7, 10] are complicated, require special equipment and are not always reproducible. We have devised a method which enables the total nitrogen to be determined in quantities of 0.5 to 10 μg in one histological section of tissue, based on the principle put forward by Hawes and Skavinski [6].

Reagents and Apparatus. An incineration mixture [5] is prepared by heating concentrated sulfuric acid, containing a 10% suspension of metallic Se, at a temperature of 290° until complete decolorization and translucency is obtained.

The following reagents are also required: a 25% solution of NaOH; a 0.1 M solution of KH_2PO_4; a 0.01 N solution of HCl; a 0.1% solution of bromcresol green in 20% ethyl alcohol (reagent A); a 0.05 m buffer of borax-succinic acid at pH = 4.5 (reagent B); a mixture of reagents A and B in a ratio of 1:9 and a mixture of water and reagent A in a ratio of 9:1.

All solutions are made up in double-distilled water; the KH_2PO_4, borax and succinic acid are twice recrystallized and made up to constant weight.

In order to measure accurately small volumes of solutions, we used a semiautomatic pipette of the Levy [8] type, calibrated gravimetrically. Otherwise we used ordinary 0.1 and 0.2 ml micropipettes.

Mineralization and subsequent diffusion were carried out in the same vessel, made from pyrex glass (Fig. 1). During diffusion and vessel was covered with a ground glass stopper.

Drops of the solution which absorbs the diffusing ammonia are held in a spiral coil, 1.5 mm in diameter, made of platinum wire, 0.2 mm in diameter. The distance between the turns and the length of the spiral are so adjusted that the volume of the drop is approximately 10 μl. The spiral is fixed to a capillary tube by means of paraffin wax.

When not in use, the platinum spirals are kept in double-distilled water, and immediately before the experiment they are cleaned by being dipped three times in boiling double-distilled water, and then flamed.

Mineralization was carried out on a sand bath, 1 cm wide, 30 cm long and 2 cm deep, heated by means of a nichrome coil buried in the sand. The heat generated by the coil was regulated by a rheostat.

In order to fix the vessels on the sand bath, clips are used, made of wire bent in the shape of round rings and attached to the sand bath, parallel to it and at a height of 1.5 cm from the surface of the sand.

Titration of the ammonia by the direct method [9] was carried out with the aid of an ultramicroburette (UMB) (Fig. 2).

From the number of ultramicroburettes described in the literature [2, 7] we selected the capillary UMB constructed by the method suggested by Korenman and Gronsberg [1, 2].

Solutions are drawn into and expelled from such a UMB by means of a pneumatic regulator. Estimation of the volume expelled is done by means of a scale, graduated in 0.5 mm. The UMB is calibrated gravimetrically. The total volume of the UMB is 17.8 μl, and one scale division corresponds to 0.024 μl. All the parts of the UMB are fixed to a panel of organic glass.

The solution to be titrated is poured into a glass dish with a diameter of 9 mm and a depth of 12 mm. By means of a glass rod, fused to it, mounted in a rubber stopper, the dish is fixed to a pedestal covered with opaque glass. The pedestal is supplied with a vertical screw transmission and a system of hinges which permit its movement in the horizontal and vertical directions.

Mixing of the fluid during titration is achieved by an electromagnetic mixer, consisting of a thin glass rod and a nonsparking electric bell AZ-127, from which the lid is removed as a preliminary measure. The glass rod is joined at one end to the handle of the striker of the bell, and the other end is dipped into the fluid to be titrated to the level of the tip of the capillary UMB.

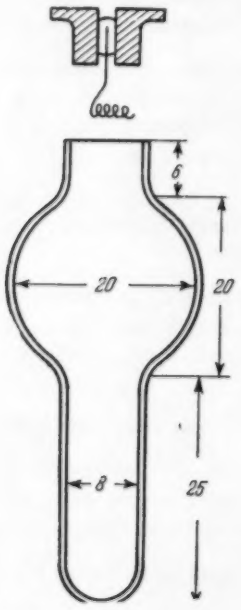

Fig. 1. Vessel for incineration of the tissue and diffusion (measurements given in mm).

The UMB, titration pedestal and electromagnetic mixer are mounted on a metal laboratory stand.

Procedure. A histological section of fresh tissue, 20-30 μ thick and 1-2 mm^2 in area, obtained with a freezing microtome, is placed at the bottom of the vessel illustrated in Fig. 1, and to it is added 18-20 ml of the incineration mixture from an ordinary 0.1 ml micropipette with a drawn out capillary delivery tube.

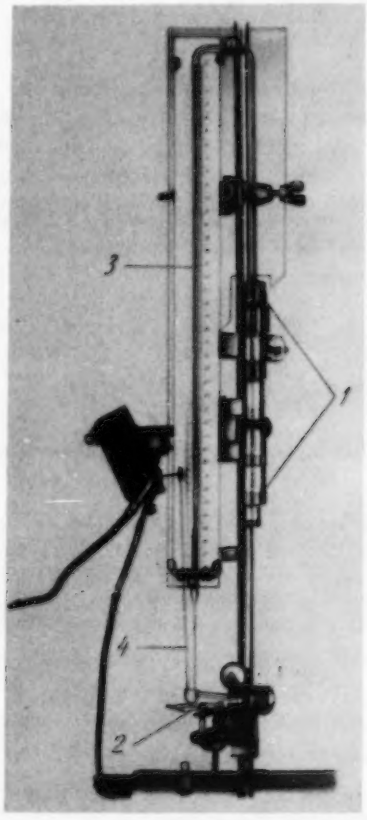

Fig. 2. Apparatus for titration. 1) Pneumatic regulator; 2) pedestal for titration with the dish fixed to it; 3) capillary tube of the ultramicroburette; 4) rod of the mixer.

The vessel is transferred to one of the wire rings so that its base is lightly buried in the sand, and inclined at an angle of about 60°.

Mineralization is continued for 4-5 hours at 255-260°, after which the vessel is allowed to cool. This time is used for preparation of the spiral coil.

The stopper of the tube is grasped with forceps and passed a few times over the flame of an alcohol lamp, in which it is heated until the paraffin wax in the capillary tube begins to melt. With another pair of forceps the platinum wire is carefully inserted into the capillary tube, the paraffin wax is allowed to coll, and the ground surface of the stopper is then smeared with a thin layer of vaseline. The stopper is turned with the spiral uppermost and left for subsequent manipulation.

After the spiral has been prepared, 0.2 ml of water is added to the cooled vessel and mixed, and the vessel is placed at an angle of 15-20° so that all the contents of the vessel are transferred to its narrow part.

Determination of the Total Nitrogen in a Standard (0.4 mg/ml) Urea Solution

Sample number	Total nitrogen content of sample (in μg)	Value determined		Error of the method (in %)
		(in μg)	(in %)	
1	1.287	1.368	106.2	+6.2
2	1.287	1.306	101.4	+1.4
3	1.287	1.336	103.8	+3.8
4	1.287	1.297	100.7	+0.7
5	1.287	1.270	98.6	−1.4
6	1.287	1.293	100.5	+0.5
7	1.287	1.269	98.6	−1.4
8	1.287	1.234	95.8	−4.2
9	1.287	1.289	100.1	+0.1

Into the spherical part of the vessel is carefully introduced 0.2 ml of 25% NaOH, the previously prepared stopper is taken and the attached spiral is immersed in a 0.1 M solution of KH_2PO_4 which, according to Hawes and Skavinski [6], possesses a higher absorbtive power than the boric acid usually used for this purpose [4, 9]. Since the absorbed ammonia is then estimated by direct titration [9], there is no necessity to measure accurately the volume of the drop, and it is sufficient to immerse the spiral once in the absorbent solution.

After withdrawl of the spiral from the solution, the vessel is quickly covered with the stopper, the fluid contained in it is thoroughly mixed by agitation, and the vessel is left in the horizontal position for 16 hours to allow diffusion and absorption of the ammonia.

After 16 hours the stopper with the spiral is removed from the vessel the spiral is carefully separated from the stopper with forceps, and placed in one piece in the titration dish, in which is 150 μl of a mixture of water and indicator solution.

Titration is performed from an ultramicroburette with a 0.01 N solution of HCl to pH = 4.5, with constant agitation of the titrated solution with the electromagnetic mixer, and with observer control (150 μl of a mixture of 0.05 M buffer of borax and succinic acid and indicator solution, in proportion of 9:1). The color change of the indicator was observed from above, against the opaque glass of the pedestal.

Each experiment must consist of a minimum of three parallel experimental and three control tests.

In order to test the completeness of mineralization, subsequent diffusion and absorption of ammonia, the total nitrogen content of a standard urea solution (0.4 mg/ml) was determined. In each sample 6.9 μl of urea solution was taken, which was equivalent to 2.76 μg urea or 1.287 μg nitrogen. In the control samples and urea solution was replaced by an equal volume of water.

Analysis of the samples of standard solution (see table) gave perfectly satisfactory results.

SUMMARY

The author describes the ultramicromethod in total nitrogen determination which makes it possible to analyze histological tissue sections (20 to 30 μ thick and with the area of 1-2 mm^2) to within the accuracy limits of ± 5-6% with the total nitrogen content in them comprising 0.5-10 ± 5-6%.

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THE INFLUENCE OF THE METHOD OF COMPRESSION ON THE VALUE OF THE TERMINAL MAXIMAL INTRA-ARTERIAL PRESSURE

L. N. Deryabin

From the I. M. Sechenov Institute of Evolutionary Physiology (Director — the late Academician L. A. Orbeli) of the AN SSSR, Leningrad

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Several investigations [1-4, 6-9 and others] have been devoted to the further study of problems associated with bloodless methods of determination of the values of the individual parameters of the intra-arterial pressure. In particular, a relationship has been shown between the terminal maximal pressure and the duration of the compression, variations in the structure of the cuff, the position and thickness of the organ on which measurements were carried out. In some investigations [1-3, 10 and others] attention was directed toward the great discrepancy between values obtained by indirect and direct measurement, reaching 50 mm Hg and more. Individual authors have observed such an increase in the values obtained by indirect measurement when using different methods of testing intravascular patency (auditory, palpatory and visual).

It is therefore of interest to examine some errors of bloodless measurement with the use of different methods of compression, paying attention to the factor of resistance of the soft tissues surrounding the artery.

EXPERIMENTAL METHOD

Experiments were carried out on dogs by means of a specially developed technique, permitting sphygmography to be done by a bloodless method and determination of the terminal maximal pressure (TMP) in the central caudal artery to be made. In order to obtain the value of the TMP, compression was brought about by means of an elastic cuff (10 × 3 cm), a rigid concentric capsule as described by V. M. Chernov [5], or a capsule for exerting local pressure on the vessel. The latter was applied to the ventral surface of the tail, where the caudal artery for a certain distance is covered only by skin and subcutaneous cellular tissue. To avoid increasing the values of the TMP as a result of displacement of the capsule from the tail in the process of compression, a second capsule was applied to the dorsal surface of the tail. The two capsules were firmly joined together and were simultaneously inflated with compressed air at the same pressure. In some experiments a cuff and a concentric capsule were applied simultaneously to the animal's tail, so that their centers were 3 cm apart. The transmitter of the sphygmographic part of the apparatus was placed distal to one or both compressing devices. The arrangement of the one or two compressing devices along the length of the tail was the same in all the experiments. The sphygmograms obtained were used for observations on the degree of intravascular patency during the determination of the TMP. The pressure was read in mm of mercury.

We carried out 86 experiments on 2 dogs. In 43 experiments the TMP was determined with the aid of a cuff, in 14 by the use of a concentric capsule, in 15 with a device for local pressure on the vessel, and in 14 experiments the TMP was measured alternately by the cuff and concentric capsule. In the course of each experiment from 10 to 20 measurements were taken.

EXPERIMENTAL RESULTS

Preliminary observations showed changes in the values of the TMP in the course of the experiment and their approximate equality at the beginning of each experiment. When the mean values were being deduced in

those cases when one compressing device was used, we therefore took note of the results of the first measurements in individual experiments. If two different compressing devices were used alternately, the mean values were calculated from all the measurements in the particular experiment. The experimental results showed that

TABLE 1

Comparison of the Values of the TMP as Determined by the Cuff and Capsule

Cuff applied to the proximal part of the tail, capsule distal to the cuff				Capsule applied to the proximal part of the tail, cuff distal to the capsule			
minutes of the experiment	values of the TMP (in mm Hg) as determined by the		excess of the value of the TMP determined by the cuff	minutes of the experiment	values of the TMP (in mm Hg) as determined by the		excess of the value of the TMP determined by the cuff
	cuff	capsule			cuff	capsule	
1	140	–	18	1	–	126	16
2	–	122		2	142	–	
3	139	–	16	3	–	121	11
4	–	133		4	132	–	
5	142	–	25	5	–	120	21
6	–	117		6	141	–	
7	148	–	22	7	–	120	14
8	–	126		8	134	–	
9	137	–	21	9	–	126	10
10	–	116		10	136	–	
11	134	–	19	11	–	125	7
12	–	115		12	118	–	
Mean value	140	122	18	–	134	123	11

the TMP, determined by means of the cuff, was 138 mm; the TMP obtained by means of the concentric capsule was 123 mm, and by means of the device for local pressure on the vessel 94.6 mm. In Table 1 are shown the results of 2 experiments carried out on a dog in which the TMP was determined by two compressing devices alternately.

TABLE 2

Comparison of the Mean Values of the TMP (in mm Hg) from All Measurements in 4 Experiments Performed with the Use of the Cuff and Capsule

Experiment No.	TMP determined by the cuff situated		TMP determined by the capsule situated	
	proximally	distally	proximally	distally
1	147.6	–	–	120.5
2	–	135	126.7	–
3	–	141	127.0	–
4	140	–	–	121.0
Mean value	143.8	138	126.8	120.7
Difference in values in the two positions	5.8		6.1	

In Table 2 are shown the results of the most demonstrative experiments carried out under analogous conditions on another dog.

The determination of the values of the TMP in the caudal artery of the dog enabled the effects of different forms of compression to be compared, and also their relationship to the resistance of the soft tissues. Experiments in which only one compressing device was used showed that the TMP obtained by the use of an elastic cuff (138 mm Hg) was 15 mm higher than that obtained by the use of a concentric capsule (123 mm Hg), and 43 mm higher than the TMP determined by local pressure on the vessel (94.6 mm Hg). In the last case the counteraction of the soft tissues to the compressing force consisted only of the resistance of the vessel wall and skin covering it. With concentric compression of the vessel, in addition to the surrounding soft tissues was added the resistance of the muscles and tendons of the tail. This addition to the counteraction of the compressing force was shown by the increase in the TMP determined by the capsule, by 28 mm Hg, and measured by the cuff by 43 mm.

In the experiments in which two compressing devices were used simultaneously (see Tables 1 and 2) a relationship was revealed between the above-mentioned excess of pressure and the soft tissue resistance. When the compressing device was applied distally and proximally, the thickness of tissue compressed, by virtue of the conical shape of the tail, differed by approximately 18%. As a result of this, the values obtained when the compressing device was situated distally were reduced: by 5.8 mm for measurements with the cuff, and by 6.1 mm for measurements with the capsule. Hence, by a rough calculation, the resistance of the whole mass of soft tissues at the point of measurement may be determined. If the thickness of the diaphyseal part of the vertebra in the region where the compression was applied accounts for 20% of the cross section of the tail, then to overcome the resistance of all the soft tissues requires about 26.5 mm Hg. The TMP with local pressure on the vessel (94.6 mm) plus the calculated value of the resistance of the soft tissues (26.5 mm) amounts to 121 mm. This value is close to that obtained in experiments using the concentric capsule, and is 17 mm less than the TMP determined by the cuff in the corresponding position of the tail (138 mm).

On the basis of the experimental findings described it may be concluded that the values of the terminal maximal intra-arterial pressure by bloodless measurement are increased by the resistance of the soft tissues surrounding the caudal artery of the dog to the compressing force. The degree of this increase depends on the mode of compression used.

SUMMARY

The terminal maximal intra-arterial pressure was determined in the tail arteries of dogs with the aid of an elastic cuff, concentric capsule and a device for exerting local pressure on the vessel. Different compression devices were used separately and in pairs, with the change of their location on the tail in conformity with the difference in the thickness of soft tissues subjected to compression. It was experimentally demonstrated that the compression measurement of the terminal maximal blood pressure is accompanied by some exaggeration of the data received. This exaggeration is connected with the resistance of the soft tissues, the extent of which depends on the method of compression.

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A METHOD OF STIMULATION OF THE PRESSURE RECEPTORS OF THE CAROTID SINUS REGION WHILE PRESERVING THE NATURAL BLOOD SUPPLY

R. M. Lyubimova (Moscow)

Scientific Director – Corresponding Member AMN SSSR Prof. A. V. Lebedinskii

(Received May 16, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

In present-day physiology several methods are available for investigating the depressor reflexes from the pressure receptors of the carotid sinus region. The most widespread method is that of E. A. Moiseev, which is based on total isolation of the carotid sinus region followed by perfusion of the sinus with a nutrient fluid. Stimulation of the pressure receptors is effected by increasing the pressure of the fluid passing through the sinus. The main defect of this method is the artificial nutrition of the receptor zone, which creates abnormal conditions of pressure and chemical composition of the fluid medium acting on the receptor apparatus; this, especially in pathological conditions, may affect the performance of the reflex.

In 1953, I. R. Petrov and A. A. Zor'kin [2] suggested a method of stimulation of the pressure receptors by means of an obturator. The introduction of an obturator into the region of the carotid sinus disturbs the normal blood supply of the receptor zone (only retrograde flow of the blood is possible, and the pressure in this region falls sharply). In addition it is quite difficult to place the obturator accurately in relation to the receptors.

We propose a method of investigation of the pressure receptors while preserving the natural blood supply, by means of a cannula and tap which is inserted into the common carotid artery.

The cannula and tap (Fig. 1) consists of a brass three-way tube with a three-way tap.

The experiment is conducted as follows: in a rabbit, previously having been given injections of heparin, under local anesthesia of the skin, the three-way tap and cannula in inserted into the common carotid artery below its division into internal and external. All the branches above the division, with the exception of one branch of the external carotid artery, are ligated (Fig. 2).

The natural blood supply is preserved when the tap is in position I. The tap is turned into position II in order that a small quantity of blood (1-1.5 ml) should reach the initial part of the system for injecting fluid, which is filled with warm physiological saline from a Dewar flask before the experiment. The admission of the sample of blood is controlled by means of the glass tube, and a clamp is applied to the unligated branch of the external carotid artery. Temporary isolation of the carotid sinus region is thus created.

The tap is next turned to position III. By means of the syringe 2 the required pressure is created in the isolated part of the carotid artery. The pressure is recorded by means of the mercury manometer 3.

After a single stimulation the blood supply is restored by turning the tap to position I. All the manipulations last for about a minute.

This method enables the state of the pressure receptors of the carotid sinus region to be studied while preserving the natural blood supply, and it does not require complicated apparatus.

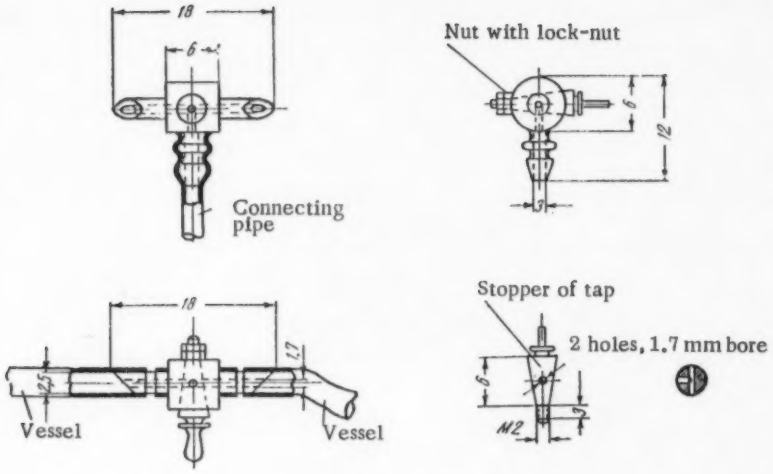

Fig. 1. Three-way tap and cannula and its scheme.

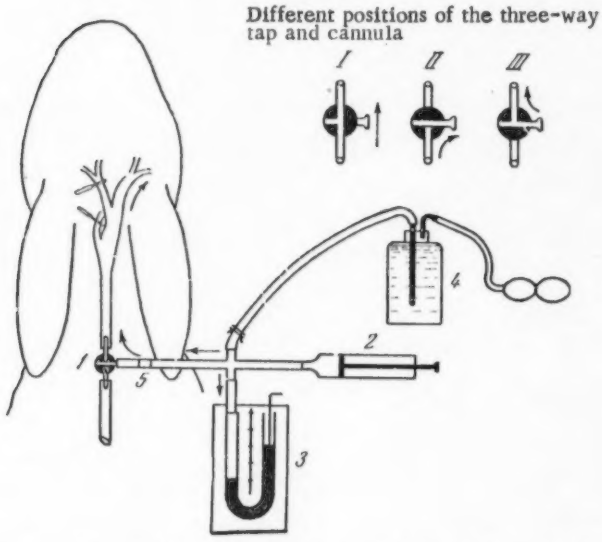

Fig. 2. Scheme of setting up the experiment with natural blood supply to the carotid sinus. 1) Three-way tap and cannula; 2) syringe; 3) mercury manometer; 4) Dewar flask; 5) glass tube; I, II, III) different positions of the three-way tap and cannula; clamp.

SUMMARY

The method presented in this paper makes it possible to investigate the baroceptors of the sinocarotid zone in conditions of natural circulation. For this purpose a 3-way tap and cannula is employed which is connected to the common carotid artery. This method does not require complicated equipment, and has many advantages over the formerly existing ones.

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LÉM	Laboratory for Experimental Morphogenesis
LÉNDVI	Leningrad Inst. of Dermatology and Venereology
LÉO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIÉM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

*BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Ekspti. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
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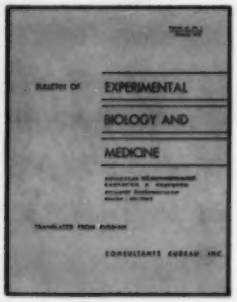

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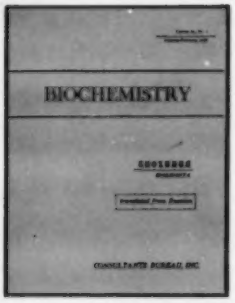

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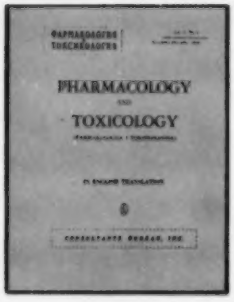

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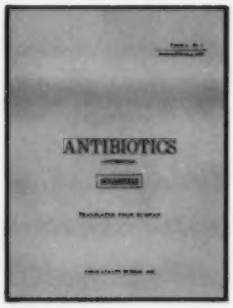

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